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THE WOODS HOLE OCEANOGRAPHIC INSTITUTION
Report for the Year 1933

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THE HAEMOCYANINS

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(*Received* August 6, 1933.)

As yet we know comparatively little about haemocyanin, especially with regard to its relation to oxygen. It is a matter which would well repay investigation to determine whether it has the remarkable properties which haemoglobin has in this respect, properties which are at present unique.

(Bayliss, *Principles of General Physiology*, 1915.)

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[1] Contribution No. 22 Woods Hole Oceanographic Institution.

I. INTRODUCTION.

THE haemocyanins are proteins occurring in solution in the blood of certain invertebrates. Together with the haemoglobins, chlorocruorins, and haemerythrin, the haemocyanins comprise a group of substances unique in uniting with oxygen in a way so labile that they serve in the transport of oxygen by the blood. Unlike the other members of this group, the haemocyanins are characterised by the presence of copper as part of their composition. The property of combining reversibly with oxygen apparently depends upon a prosthetic group of which the copper forms an essential part.

The earlier literature relating to the haemocyanins was reviewed by Quagliariello (1923, 1924) and Dhéré (1928). Subsequently a considerable volume of precise work has appeared which not only corrects or extends many of the earlier observations, but contributes to a number of questions scarcely raised by the earlier workers. At the present time our knowledge of the haemocyanins compares favourably in many respects with that of the haemoglobins. The purpose of the present review is to collect these recent data which define the properties of this group of substances.

Haemocyanins occur in the blood of molluscs and arthropods. Detailed studies have been made on haemocyanins isolated from the cephalopods (*Octopus*, *Sepia*, *Loligo*), the gastropods (*Helix*, *Busycon*), the decapod Crustacea (*Cancer*, *Homarus*, *Maia*, *Palinurus*, etc.) and the Xiphosura (*Limulus*). The blood of certain spiders appears to contain haemocyanin (Wilson, 1901), and Svedberg and Hedenius (1933) have confirmed Lankester's (1884) observation that this substance occurs in the blood of scorpions. Cuénot (1891) lists a number of gastropods and lamellibranchs of which the blood is considered to contain haemocyanin, but the identity in these cases is not established by isolation or chemical analysis, but rests merely on the blue colour of the body fluids. Svedberg and Hedenius have examined the blood of a great number of lamellibranchs by means of the ultra-centrifuge without finding haemocyanin.

It has been suggested by Muttkowski (1921) on the basis of the qualitative demonstration of copper in the blood of certain insects, that these contain haemocyanin. We have measured the concentration of copper in the blood of the Florida grasshopper and have found quantities so small that if it is present as haemocyanin the latter can have no importance in the transport of oxygen by the blood.

In no case is haemocyanin known to occur in blood corpuscles or within tissue cells.

II. THE CHEMICAL PROPERTIES OF HAEMOCYANIN.

(1) *Chemical composition.*

The best data on the elementary composition of the haemocyanins are given in Table I. They are based on the analyses of Hernler and Philippi (1930, 1933), made upon materials purified by the best available methods. The copper contents shown by these analyses agree closely with the earlier measurements made upon

Table I. *Elementary composition of various haemocyanins.*

	C %	H %	N %	S %	Cu %
Gastropods					
Helix pomatia	53·4	6·9	15·15	0·76	0·24
Busycon canaliculatum	53·5	6·65	15·9	1·23	0·245
Cephalopods					
Octopus vulgaris	53·4	6·95	15·9	1·04	0·25
Loligo pealei	52·75	6·8	15·75	1·19	0·26
Xiphosura					
Limulus polyphemus	53·4	6·9	16·9	1·10	0·173
Crustacea					
Homarus americanus	53·07	6·85	16·78	0·90	0·187

the haemocyanin of *Helix* by Dhéré (1919*b*), Burdel (1922), and Begemann (1924); of *Limulus* by Redfield, Coolidge and Shotts (1928); of *Busycon* and *Loligo* by Montgomery (1930). Earlier analyses of the copper content of the haemocyanin of *Limulus* by Alsberg and Clark (1910), of *Octopus* by Henze (1901), and of *Sepia*, *Cancer* and *Homarus* by Griffiths (1892) cannot be reconciled with these measurements; the determinations of the other elements made by these authors do not deviate widely from those of Hernler and Philippi.

The general proportions of C, H, O, N and S in the haemocyanins are a reflection of their protein nature. It is noteworthy that the haemocyanins of the arthropods, *Limulus* and *Homarus*, contain definitely more nitrogen than do those of the molluscs. A similar distinction occurs in the case of the copper content—the arthropod haemocyanins containing about 0·18 per cent., whereas the molluscan haemocyanins yield approximately 0·25 per cent. copper. Were it not for the distinct differences in the physical properties of the haemocyanins within each of these groups, one might conclude that within each phylum the haemocyanins were similar, if not identical.

The haemocyanin of *Busycon canaliculatum*, which was described by Mendel and Bradley (1907) under the name *haemosycotypin*, was thought by these authors to contain zinc as well as copper. Recent spectrographic analysis of this, as well as other species of haemocyanin by Gatterer and Philippi (1933), has failed to confirm the presence of zinc or of any other heavy metal except copper in purified preparations of the proteins. Unpublished experiments made by Dr Beecher in the author's laboratory were also unsuccessful in demonstrating the presence of zinc in purified preparations of *Busycon* haemocyanin, although this element could be detected in preparations of whole blood.

Further knowledge of the composition of the haemocyanins is fragmentary. Henze (1904) estimated the distribution of nitrogen in the haemocyanin of *Octopus vulgaris* to be as follows:

	%
NH_3 nitrogen	5·78
Humin nitrogen ...	2·67
Diamid nitrogen ...	27·65
Monoamid nitrogen ...	63·39

Van Slyke (1911–12) obtained the following results from a study of the distribution of nitrogen in *Limulus* haemocyanin:

	%
NH_3 nitrogen	5·95
Melanin nitrogen	1·65
Cystine nitrogen	0·80
Arginine nitrogen	15·73
Histidine nitrogen	13·23
Lysine nitrogen	8·49
Amino nitrogen in filtrate	51·30
Non-amino nitrogen in filtrate (prolin, oxyprolin, ½ tryptophane)	3·80

Sulphur occurs in all haemocyanins in quantities which appear to vary from species to species. Prof. H. T. Clarke (personal communication) has found that less than half of the sulphur in *Limulus* haemocyanin may be accounted for as cystine sulphur.

(2) *The prosthetic group.*

Particular interest attaches to the chemical nature of the portion of the haemocyanin molecule which contains the copper and which presumably functions as an oxygen carrier. The phenomenal success made by students of this problem in the case of haemoglobin has stimulated an analogous investigation of the prosthetic group of haemocyanin.

Henze (1901) observed that treatment of *Octopus* haemocyanin with acid led to a separation of the copper from the protein. He concluded that the copper was not present in a stable organic binding, as is the iron in haemoglobin, but is present as a copper albuminate. Henze's view was supported by Kobert (1903) and Alsberg and Clark (1910). Roche (1930) has also concluded from a comparison of the physical properties of the protein before and after removal of the copper by treatment with acid that one cannot accept the presence in haemocyanin of a prosthetic group analogous to that of haemoglobin, but more recently he appears to have abandoned this view (Roche and Dubouloz, 1933).

On the other hand, by adopting the methods used in separating the phyllins of chlorophyll, Philippi (1919) secured from *Helix* haemocyanin treated with strong alkali a dark green precipitate which contained 7 per cent. copper. This product gave an intense pyrrol reaction with the pine splint test. The result was of great interest, as pyrrols form the basis of the structure of the prosthetic group of haemoglobin. Dhéré and Schneider (1922*a*) also prepared this product and proposed to call it *hématocyanine*. Dhéré and Baumeler (1928, 1929) were unable to detect in this product any derivatives giving the red fluorescence characteristic of porphyrins, as would be expected were the prosthetic group of haemocyanin similar to that of haemoglobin.

Schmitz (1930, 1931*a*, *b*) secured a product by decomposing *Octopus* haemocyanin with alkali which he designated *Hämocuprin*. This substance has the composition C 45·04, H 6·99, N 12·58, Cu 6·27 per cent. He believes it to be a tetrabasic organic acid containing copper in complex binding, and finds no evidence of porphyrin structure.

Conant and Humphrey (1930) likewise secured an insoluble copper compound by treating *Limulus* haemocyanin according to Philippi's procedure. After further purification a black amorphous powder containing practically all the copper of the original haemocyanin was obtained. This product had the following composition:

			%
Cu	...	...	21·5
N	...	...	9·2
C	...	...	39·5
H	...	...	5·6
S	...	...	8·0

The available evidence indicated that this product is a complex salt of an amino acid which contains sulphur.

By the action of ammonia on this pigment an amorphous, colourless solid was obtained which was free from copper and sulphur and gave no evidence of containing pyrrol nuclei.

Conant, Dersch, and Mydans (personal communication) have found that this material is a polypeptide composed of leucine, tyrosine, and at least one other amino acid tentatively identified as serine. Basic amino acids, tryptophane, and histidine are absent. The sulphur in the original black powder is present neither as cystein, cystin, nor methionine. Since the sulphur compound and the copper are removed when the black material is treated with ammonia, it is probable that the sulphur compound is linked through the copper and not by a peptide linkage to the polypeptide. They suggest that the prosthetic group of *Limulus* haemocyanin is a complex copper salt of an unknown sulphur compound and a polypeptide, consisting of one molecule each of leucine and tyrosine and three of serine.

Conant and his collaborators have evidence that this complex is in reality a prosthetic group of haemocyanin, and is not formed by the combination of fragments into which the original protein is decomposed by the action of alkali. They are unable to obtain a product similar to Schmitz' *Hämocuprin* by applying his procedures to *Limulus* haemocyanin, but secure a product similar to *Hämocuprin* by prolonged treatment of their black powder with normal sodium hydroxide. It appears, therefore, that there may be some differences between *Octopus* haemocyanin and that of *Limulus* which alter the ease of decomposition of the complex copper compound by alkali, but that in both cases essentially the same sort of prosthetic group is present. Differences in the ease with which copper may be separated from different haemocyanins is also indicated by the fact that the haemocyanins of *Octopus*, *Limulus*, and *Busycon* give the biuret reaction on the addition of alkali, but that of *Helix* does not give a positive reaction unless a trace of copper is also added (Dhéré, Baumeler and Schneider, 1929).

Finally, Laporta (1932) has secured a product which he considers identical with Schmitz' *Hämocuprin* by treating *Octopus* haemocyanin with acetone and acid. This product contains copper and gives the pine splint test of pyrrol. The product obtained by this method from the haemocyanins of the octopus, crab, and snail have the same ultra-violet absorption spectrum (Roche and Dubouloz, 1933).

This is all that can be said concerning the chemical composition of the haemocyanins and the structure of the prosthetic group. From the standpoint of the organic chemist much remains to be done.

(3) *The molecular weight.*

The minimal molecular weight of the haemocyanins, estimated as the smallest weight which would contain one atom of copper, is about 25,000 in the case of the molluscan haemocyanins and 37,000 in the case of those of the arthropods. These are the largest known minimal molecular weights to be based on purely analytical data. As will be pointed out below, the quantity of haemocyanin which unites with one molecule of oxygen contains two atoms of copper. On the basis of their oxygen-binding power the minimal molecular weights of the two classes of haemocyanin become 50,000 and 74,000.

Experiments by Cohn (1925) on the filtration of proteins through collodion membranes placed *Limulus* haemocyanin as intermediate in size between haemoglobin and pseudoglobulin, which would indicate that its true molecular weight is equal to the minimal weight 74,000 or a value twice as great. The equilibrium between purified *Limulus* haemocyanin and oxygen may be described by the mass law, assuming that each molecule of haemocyanin unites with a single molecule of oxygen. This would be the case were the molecular weight 74,000.

While there is thus some evidence that the molecule of *Limulus* haemocyanin contains but two atoms of copper and has a molecular weight comparable in size to that of haemoglobin and many other proteins, other evidence suggests that the haemocyanin molecules are much larger, or at least that the units of minimal molecular weight are associated in aggregations of uniform size but of much greater bulk. Dhéré and Baumeler (1926) observed that haemoglobin would pass through collodion membranes which retained *Helix* haemocyanin and concluded that the haemocyanin molecules are larger than those of haemoglobin (molecular weight 66,800). The equilibrium of haemocyanin with oxygen, in the case of the native blood of several species, can be accounted for under certain circumstances on the assumption that the haemocyanin forms a stable oxygenation product only when it unites with four molecules of oxygen. This assumption necessitates a haemocyanin molecule containing eight atoms of copper and consequently possessing a molecular weight of 200,000 in the case of the molluscs, and 300,000 in the case of the arthropods. The equilibrium between haemocyanin and cyanide (see p. 203) and oxidation-reduction equilibria (Conant, Chow and Schoenbach, 1933) also suggest a haemocyanin unit of about this size.

The evidence of Svedberg and his collaborators, obtained by means of studies of the sedimentation velocity and equilibrium of haemocyanin solutions under centrifugal force, show that the haemocyanins may exist as particles of uniform size having the weights shown at the top of p. 181.

These are values unapproached by other proteins save for the respiratory proteins of the blood of certain worms, in which haemoglobin and chlorocruorin occur in solution (Svedberg and Eriksson, 1932*a*).

Helix haemocyanin	...	5,000,000 (Svedberg and Chirnoaga, 1928)
Octopus haemocyanin	...	2,000,000 (Svedberg and Eriksson, 1932*b*)
Limulus haemocyanin	...	1,300,000[1]
Homarus haemocyanin	...	640,000 (Svedberg and Eriksson, unpublished)
Palinurus	...	360,000 (Svedberg and Eriksson, unpublished)

Svedberg has published measurements of the sedimentation constant of the haemocyanins of a large number of species. The sedimentation constant is defined as the velocity of sedimentation in a centrifugal field of unit strength, reduced to water of 20° as solvent. This constant is a function of the mass and shape of the molecule and is thus characteristic of the molecular species studied. The sedimentation constants of the haemocyanins examined are given in Table II. These

Table II. *Sedimentation constants of various haemocyanins.*

Species	Sedimentation constant $S_{20^\circ} \times 10^{13}$	Species	Sedimentation constant $S_{20^\circ} \times 10^{13}$
Crustacea		Gastropoda (*cont.*)	
Palaemon fabrici	16·9	*Littorina littorea*	99·8
Pandalus borealis	16·9	*Buccinum undatum*	99·8
Palinurus vulgaris	16·9	*Neptunea antiqua*	99·8
Eupagurus bernhardus	16·9	*Limnea stagnalis*	99·8
Pagurus striatus	16·9	*Achatina fulva*	99·8
Nephrops norvegicus	23·4	*Helix pomatia*	99·8
Homarus vulgaris	23·4	*H. arbustorum*	99·8
Astacus fluviatilis	23·4	*H. nemoralis*	99·8
Hyas araneus	23·4	*H. hortensis*	99·8
Maia squinado	23·4	*Limax maximus*	99·8
Cancer pagurus	23·4	*L. agrestis*	99·8
Carcinus maenas	23·4	*Arion ater*	60·8
Squilla mantis	23·4	Cephalopoda	
Calocharis mâcandrae	34·1	Decapoda	
Arachnomorpha		*Loligo vulgaris*	57·1
Limulus polyphemus	35·7	*Sepiola oweniana*	57·1
Euscorpius sp.	34·1	*Rossia macrosoma*	57·1
Amphineura		*Sepia officinalis*	57·1
Tonicella marmorea	60·8	Octopoda	
Gastropoda		*Octopus vulgaris*	51·1
Paludina vivipara	99·8	*Eledone cirrosa*	51·1
P. contecta	99·8	*E. moschata*	51·1

haemocyanins are all characterised by high sedimentation constants and have therefore high molecular weights. Within each well-defined group all the species have, as a rule, practically the same sedimentation constant.

These enormous molecules, which each comprise from 20 to 80 oxygen equivalents, appear to be made up of associations of smaller units. *Helix* haemocyanin, at reactions removed from the isoelectric point, gives sedimentation velocity measurements which indicate that under these conditions the particles disintegrate into smaller particles of undetermined weight (Svedberg and Heyroth, 1929*b*). *Limulus* haemocyanin appears to disintegrate into smaller units on dilution to

[1] Prof. Svedberg informs me that recent measurements indicate that the molecular weight of *Limulus* haemocyanin is 1,300,000 rather than 2,000,000 as reported by Svedberg and Heyroth (1929*a*).

concentrations of 0·1 per cent. or less (Svedberg and Heyroth, 1929*b*). The haemocyanin of *Homarus* dissociates at high alkalinities into molecules of half the size of those which exist at neutral reactions. In *Octopus* haemocyanin there is present, both in the case of the blood and of purified preparations, two components, one of uniform molecular weight of 2,000,000, the other of smaller size. The size of the latter and the ratio of the two components both vary widely and reversibly with hydrogen-ion concentration.

(4) *Molecular dimensions and symmetry.*

From considerations involving diffusion constants, molecular weight, and viscosity Svedberg and his collaborators conclude that the molecule of *Helix* haemocyanin is spherical and has a radius of 12×10^{-7} cm. The dissymmetry number of *Helix* haemocyanin is 1·05. Prof. Svedberg informs me that recent work indicates that the molecules of *Limulus* and *Octopus* haemocyanin are also nearly spherical and that the published dissymmetry numbers for these proteins are subject to revision.

(5) *Partial specific volume.*

Svedberg and his collaborators have found that the partial specific volumes of various haemocyanins are almost identical and are independent of concentration. The values obtained are:

Helix pomatia ...	...	0·738 (Svedberg and Chirnoaga, 1928)
Limulus polyphemus	...	0·735 (Svedberg and Heyroth, 1929*a*)
Octopus vulgaris	...	0·740 (Svedberg and Eriksson, 1932*b*)

The partial specific volume of most other proteins is slightly greater than these values (Svedberg, 1930*a*).

(6) *The isoelectric point.*

The isoelectric points of the various haemocyanins obtained by the method of cataphoresis are recorded in Table III. The differences in these values indicate that the haemocyanins of the different species are distinct chemical substances. Even members of the same genus—as in *Paludina* and *Helix*—have different isoelectric points. For the genus *Helix*, containing several sub-genera, the isoelectric points lie closer together within the sub-genus.

(7) *Solubility.*

The solubility of the haemocyanins of *Limulus*, *Octopus*, and *Helix* in distilled water, after the addition of various quantities of HCl or NaOH, has been measured by Roche (1930, 1932). His results are shown in Fig. 1. Aside from this, no adequate studies have been made of the solubility of haemocyanins. Nevertheless, there exists considerable qualitative information which seems to characterise the haemocyanins of different species.

The haemocyanins all appear to be globulins (Quagliariello, 1924). They are soluble in dilute salt solutions but are insoluble at certain hydrogen-ion con-

Table III. *Isoelectric point, pH value at which solubility is minimal and slope of the mobility-pH curve* $\left(\frac{du}{dp\mathrm{H}}\right)$ *of various haemocyanins.*

Species	Isoelectric point	*p*H of minimal solubility	$\frac{du}{dp\mathrm{H}} \times 10^{-5}$	Authority
Octopus vulgaris	4·7	—	—	Quagliariello (1920*b*)
O. vulgaris	4·8	4·8–5·0	—	Roche (1930)
Eledone cirrosa	4·6	—	—	Pedersen (1933)
Helix pomatia	5·05	—	8·1	Svedberg (1930*a*); Pedersen (1933)
H. pomatia	5·3	—	—	Stedman and Stedman (1927)
H. pomatia	—	5·2	—	Roche (1932)
H. nemoralis	4·63	—	11·4	Pedersen (1933)
H. hortensis	4·57	—	12·1	Pedersen (1933)
H. arbustorum	5·50	—	7·6	Svedberg (personal communication)
Paludina vivipara	4·71	—	10·8	Svedberg (personal communication)
P. contecta	4·63	—	11·4	Svedberg (personal communication)
Littorina littorea	4·34	4·4	12·8	Svedberg (personal communication)
Buccinum undatum	4·61	4·6	13·7	Svedberg (personal communication)
Achatina fulva	5·03	—	7·8	Svedberg (personal communication)
Cancer sp.	4·7	—	—	Stedman and Stedman (1927)
C. pagurus	4·65	—	16·0	Pedersen (1933); Svedberg (personal communication)
Homarus sp.	—	4·8	—	Stedman and Stedman (1926*a*)
H. vulgaris	4·95	—	18·0	Pedersen (1933); Svedberg (personal communication)
Astacus fluviatilis	4·93	4·8	12·1	Pedersen (1933)
Limulus polyphemus	6·4	—	—	Svedberg (1930*a*)
L. polyphemus	—	6·2–6·5	—	Roche (1930)
L. polyphemus	—	6·3	—	Stedman and Stedman (1926*a*)

centrations if the concentration of electrolyte is sufficiently low. The data recorded in Table III show that the hydrogen-ion concentration at which solubility is minimal coincides with the isoelectric point, as measured by the method of cataphoresis.

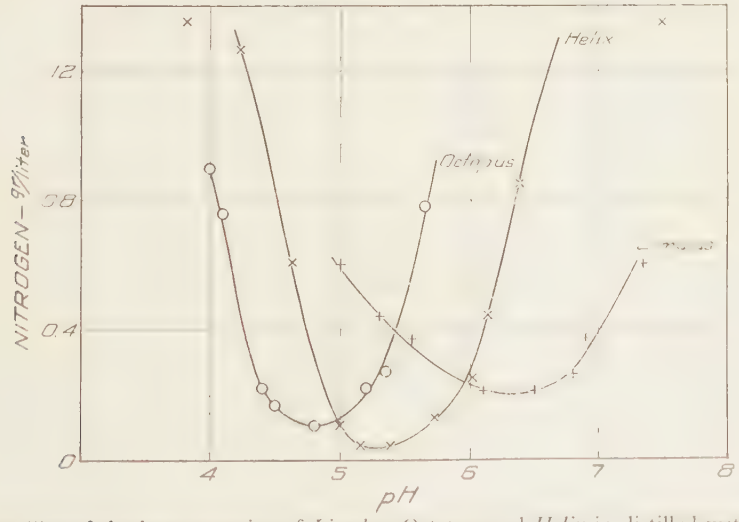

Fig. 1. Solubility of the haemocyanins of *Limulus*, *Octopus*, and *Helix* in distilled water, after the addition of various quantities of HCl or NaOH.

The various haemocyanins show great quantitative variation in the effect of salts on their solubility. *Busycon* haemocyanin may be precipitated at the isoelectric point in the presence of considerable salt, and one may isolate the protein from the blood merely by diluting the latter with a few volumes of water and then adding sufficient dilute acid. *Limulus* haemocyanin must be dialysed until almost free of salt before it can be so separated. *Helix* haemocyanin is even more soluble than *Limulus* in dilute salt solutions (Svedberg and Heyroth, 1929*a*). Pedersen (1933) observed that the haemocyanins of *Paludina vivipara*, *P. contecta*, *Achatina fulva*, *Helix pomatia*, *H. nemoralis*, and *H. hortensis* are soluble in 0·02 *M* sodium acetate buffers at the isoelectric point, whereas the haemocyanins of *Buccinum undatum*, *Littorina littorea*, and *Astacus fluviatilis* were insoluble.

Limulus haemocyanin is insoluble in the presence of salt at acid reactions (Redfield and Mason, 1928). The haemocyanins of different species appear to differ in the concentration of salt required to "salt out" the protein. *Limulus* haemocyanin is precipitated in half-saturated ammonium sulphate, whereas full saturation is required for the salting out of *Octopus* haemocyanin (Alsberg and Clark, 1910). The results of such experiments depend upon the hydrogen-ion concentration and temperature and here again extensive measurements under controlled conditions are lacking.

Craifaleanu (1918) observed that *Octopus* haemocyanin which has been kept in the crystalline condition in half-saturated ammonium sulphate for a time becomes insoluble in distilled water and proposed the name *parahaemocyanin* for this modification. The haemocyanin of the squid displays the same phenomenon (Montgomery, 1930).

(8) *Crystallisation.*

The conditions under which haemocyanin may be obtained in crystalline form are reviewed by Dhéré (1919*b*). The haemocyanin of the octopus was first crystallised by Henze (1901), employing the method of Hofmeister and the improvement introduced by Hopkins and Pinkus. On the addition of acetic acid to a saturated solution of haemocyanin dissolved in strong ammonium sulphate, the protein becomes less soluble and crystallises out. The haemocyanin of *Loligo* may be crystallised in a similar fashion. Montgomery (1930) has found that raising the temperature decreases the solubility of the haemocyanin and causes crystallisation without necessitating the addition of acid. Maximum yields were obtained by combining the two procedures.

The haemocyanin of *Helix*, which is insoluble in the absence of electrolytes, has been crystallised by Dhéré by prolonged dialysis. In another procedure an electric current is passed through a dialysed solution, causing crystallisation of the haemocyanin to occur at the positive pole. If one adds an excess of these crystals to a very dilute ($N/500$) solution of sodium sulphate a saturated solution is obtained from which an abundant crystallisation takes place on standing. Dhéré and Baumeler (1929) discovered that if papain is added to filtered *Helix* blood, or

alkaline solutions of crystalline haemocyanin, and the solutions allowed to stand, after twenty-four hours numerous needle-shaped crystals appear. These will dissolve, but with difficulty, in water, and the solutions are similar in many ways to those of the original haemocyanin. Philippi and Hernler (1930) determined the elementary composition of such crystals and concluded that they were probably pure haemocyanin, but without definitely excluding the possibility that they might be slightly modified addition or decomposition products of the original protein.

The haemocyanin of *Palinurus* may be obtained in crystalline form by a similar method. If the serum is placed in an electric field an amorphous precipitate forms at the anode. The portion of the liquid containing the precipitate is diluted with sodium chloride to a final concentration of $N/5$ which causes the precipitate to dissolve. On standing, crystals of haemocyanin are formed.

The haemocyanins of *Limulus* and *Busycon* have not been obtained in crystalline form (Dhéré, Baumeler and Schneider, 1929).

The form of the crystals is very variable, being cubic(?), quadratic, and hexagonal (or rhombic?) in the snail, cubic and rhombohedral in *Palinurus*. Those of the cephalopod haemocyanins are long needles. The form of the crystals varies greatly with the method of preparation.

The crystals of *Octopus* haemocyanin (Henze, 1901) and those of the squid are doubly refractive.

(9) *The combination of haemocyanin with acid and base.*

The amphoteric character of the haemocyanins arises from their protein nature. It is important not only as a means of chemical characterisation, but because the ability to combine with base enables the haemocyanins to perform a vital physiological function in the transport of carbon dioxide in respiration, and in buffering the blood.

Quagliariello (1916) showed that relatively large amounts of acid or alkali must be added to the blood of certain invertebrates, containing haemocyanin, in order to alter its reaction significantly. The equilibrium of the blood of a number of invertebrates with carbon dioxide was studied by Parsons and Parsons (1923), who pointed out that those bloods which combine with carbon dioxide in quantity are rich in haemocyanin. These results were confirmed in the case of *Limulus* blood by Redfield, Coolidge and Hurd (1926) and Redfield, Humphreys and Ingalls (1929). The latter conclude that substances other than haemocyanin account for only a small part of the buffering of *Limulus* blood.

Nitzescu and Cosma (1927), on the other hand, measured the carbon dioxide dissociation curve of the blood of *Helix pomatia*. They observed that although the amount of carbon dioxide bound was high, the blood was a weaker buffer than rabbit serum, which contains an equivalent amount of protein. They concluded that proteins do not play the principal rôle in the acid-base equilibrium of *Helix* blood.

The haemocyanin of *Limulus* binds about 160×10^{-5} mol of acid per gram.

The total base-binding capacity has not been precisely determined, but is of a similar magnitude. Redfield and Mason (1928) pointed out that the acid-binding power agrees closely with that calculated from the dibasic amino acids present in its composition. Roche (1932) has shown that the haemocyanin of *Helix pomatia* binds 91×10^{-5} mol of HCl and 61×10^{-5} mol of NaOH per gram, quantities notably inferior to those characterising *Limulus* haemocyanin. The lower diamino-acid content suggested by this result is possibly correlated with the lower nitrogen content of *Helix* haemocyanin as compared with that of *Limulus*.

From the oxygen-binding power of the haemocyanins it may be estimated that a quantity of the protein which combines with one molecule of oxygen contains in the case of *Limulus* 117 groups capable of neutralising HCl. In the case of *Helix* the equivalent acid-combining capacity is 45, the equivalent base-combining capacity 30.

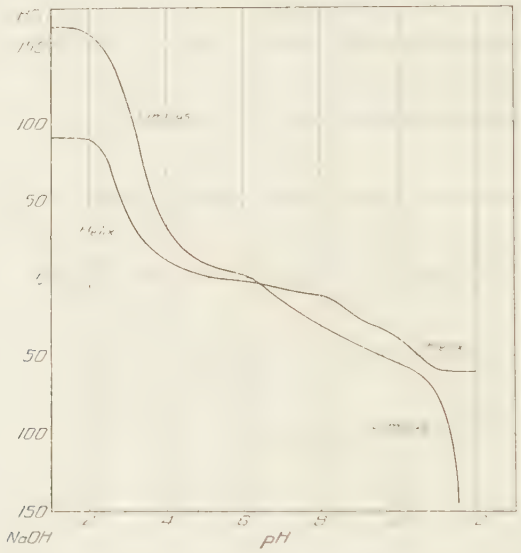

Fig. 2. Titration curves of the haemocyanins of *Helix* and *Limulus*. Ordinates, mols of HCl or NaOH added per 10^5 grams protein.

Complete titration curves of the haemocyanins of *Limulus* and *Helix*, based on the measurements of Redfield and Mason (1928), Redfield, Humphreys and Ingalls (1929), and Roche (1932), are shown in Fig. 2. These curves show certain individual differences as do the less complete titration curves for the haemocyanins of *Homarus vulgaris* (Stedman and Stedman, 1926*a*), of *Busycon canaliculatum* (Redfield and Ingalls, 1932), and the octopus (Roche, 1930). They are, however, similar in form to the titration curves of other proteins such as those published by Cohn (1925).

Limulus haemocyanin reacts with acid as though it were a univalent base with a pK value of 3·3 (Redfield and Mason, 1928); for *Helix* the pK value in the acid range is 3·2 (Roche, 1932). The titration curves in the alkaline range are more

complicated because, apparently, the various base-binding groups differ in the values of their dissociation constants. Roche considers that three dissociation constants may be distinguished in *Helix* haemocyanin characterised by pK values of 10·5, 8·6 and 7·6. The latter value changes to 7·0 when the haemocyanin becomes oxygenated. In *Limulus* haemocyanin the greater part of the alkali is bound at reactions in the neighbourhood of pH 11. Considerable buffer action is observed at more neutral reactions corresponding to the lower pK values distinguished by Roche.

The low base-binding capacity of *Helix* haemocyanin in the physiological range, as compared to that of *Limulus*, accounts for the poor buffering observed by Nitzescu and Cosma (1927) in the blood of the snail.

The individuality of the various haemocyanins in regard to their acid-base-binding power is brought out by the way in which the mobility of the protein in an electric field varies with pH in the neighbourhood of the isoelectric point. The values of the slope of the mobility-pH curve $\left(\frac{du}{dp\mathrm{H}} \times 10^{-5}\right)$ given in Table III are based on the measurements of Pedersen (1933) and on unpublished values kindly given by Prof. Svedberg.

The titration curve of *Limulus* haemocyanin does not coincide with that of the serum of this animal. The addition of sodium chloride to the haemocyanin alters the titration curve of *Limulus* and *Busycon* haemocyanin in such a way as to indicate that the pK values of the base-binding groups are decreased about 0·5 pH unit (for the addition of 0·5 M NaCl). Similar effects have been observed in the case of other proteins (Sørensen, Linderstrøm-Lang and Lund, 1927; Simms, 1929). By dissolving the haemocyanin in a mixture of salts approximating to that found in the serum a titration curve was obtained closely resembling that of the serum. It was on the basis of this observation that it was concluded that the haemocyanin was the principal buffer of the *Limulus* blood.

The buffer value of reduced *Limulus* haemocyanin is nearly constant over the pH range from 6·0 to 10 and amounts to 0·15 mol per gram of protein. This value is nearly the same as that of reduced haemoglobin which varies between 0·143 and 0·169 in different species (Redfield, 1933). The addition of salt increases the buffer value of *Limulus* haemocyanin to about 0·23 mol per gram in the physiological range of hydrogen-ion activity.

The pK values of some base-binding groups of certain haemocyanins are also altered by oxygenation. This phenomenon is analogous to that observed in many species of haemoglobin. Kerridge (1926) found in studying the carbon dioxide dissociation curves of the blood of *Maia squinado* that the oxygenated haemocyanin was a stronger acid than the reduced haemocyanin. As a result, in the range of pH 6·3–6·9 the reduced blood combined approximately 0·5 millimol more carbon dioxide per litre than did the oxygenated blood at the same pH value. She estimated that in this range the quantity of carbonic acid displaced on oxygenation was equivalent to the quantity of oxygen bound. Qualitatively similar results have been obtained by Parsons and Parsons (1923) with the blood of

Octopus macropus and by Redfield, Coolidge and Hurd (1926) with that of *Loligo pealei*.

In discussing the analogous phenomenon displayed by haemoglobin, Henderson (1920) pointed out that it followed as a physical necessity that since carbon dioxide (or other acids) diminishes the affinity of haemoglobin for oxygen, the combination of oxygen with haemoglobin must reduce the tendency of the blood to take up carbon dioxide. The foregoing observations on the haemocyanins of *Maia*, *Octopus*, and *Loligo* are in conformity with this consideration since it has been shown, in the case of *Maia* (Hogben, 1926) and *Loligo* (Redfield, Coolidge and Hurd, 1926), that the oxygen equilibrium is shifted by changes in acidity in a manner similar to that of haemoglobin.

The bloods of *Limulus*, *Busycon* (Redfield, Coolidge and Hurd, 1926) and *Helix* (Hogben, 1926) differ from those of the Crustacea and cephalopods and from haemoglobin in that at physiological reactions the affinity of the haemocyanin for oxygen is increased by the addition of acid. From the principle set forth by Henderson one would expect these bloods to combine with more carbon dioxide (or other acid) when oxygenated than when reduced. This result is obtained with *Busycon* serum. In the case of *Limulus* no difference could be detected in the carbon-dioxide dissociation curves of oxygenated and reduced blood. Studies by Mr Shack of the complete titration curves of the serum and of the purified haemocyanin of *Limulus* have failed to show any difference in the behaviour of oxygenated and reduced samples. It is possible that the effect expected on theoretical grounds is present but is too small to be detected.

The titration curves of the oxygenated and reduced haemocyanin of *Helix* have been examined by Roche (1932), who concludes that the base-binding groups having a pK value of 7·6 in the reduced haemocyanin possess a pK value of 7·0 when the protein is oxygenated. Oxygenation thus makes the haemocyanin appear to act like a stronger acid—as it does with haemoglobin. This result is not what one would expect from the data on the oxygen equilibrium at various reactions, for Stedman and Stedman (1928) concluded that purified *Helix* haemocyanin was uninfluenced in its affinity for oxygen by alterations in hydrogen-ion concentration. A further analysis of their data led Redfield (1930*b*) to suggest that increasing acidity favoured the union of *Helix* haemocyanin with oxygen—a result directly opposed to the expectation raised by Roche's observations on the titration curves.

By making the assumption that the difference in the titration curves of oxygenated and reduced haemocyanin is due to a shift in the pK value of a single acid-binding group for each molecule of oxygen combined by the haemocyanin, Mr Shack has calculated the change in the value of the pK of this group. With the exception of *Helix* haemocyanin, the anomalous behaviour of which is pointed out above, the magnitude of this change parallels the magnitude of the change in oxygen equilibrium brought about by altering the hydrogen-ion activity. This parallelism is illustrated in Table IV, in which the change in pK due to oxygenation is compared with the maximum change in oxygen pressure required to half-saturate the haemocyanin which may be produced by altering the acidity of the solutions.

Table IV. *Comparison of the change in pK (ΔpK) of the acid-binding group of haemocyanin and haemoglobin influenced by oxygenation, and the change in oxygen pressure (Δp_{50}) required to half-saturate the protein due to altered acidity.*

	ΔpK	Δp_{50}
		mm. Hg
Lobster haemocyanin		
Native serum (Shack, 1933)*	0·758	—
Dialysed serum (Stedman, 1926*a*)	—	370
Maia haemocyanin		
Native serum (Kerridge, 1926)	0·50	—
,, ,, (Hogben, 1926)	—	71
Horse haemoglobin		
Dialysed	0·35	17
Limulus haemocyanin		
Purified haemocyanin (Shack)*	0	—
,, ,, (Redfield, 1930*b*)	—	− 3
Native serum (Redfield and Ingalls, 1933)	—	−12

* Unpublished data.

(10) *Viscosity.*

Observations on the viscosity of the haemocyanins of *Cancer* and *Homarus* have been made by Stedman and Stedman (1927). Their conclusion that a relation exists between the viscosity and the affinity of the protein for oxygen has been discussed by Redfield and Ingalls (1932). Svedberg and Heyroth (1929*b*) suggest that the change in viscosity of haemocyanin solutions may be due in part at least to the dispersion of the protein into smaller units such as they observe in *Helix* haemocyanin at reactions removed from the isoelectric point.

III. OPTICAL PROPERTIES.

(1) *Index of refraction.*

Quagliariello (1920*a*) has made a systematic investigation of the refractive index of solutions of *Octopus vulgaris* haemocyanin. He found that the refractive index of the solution is increased over that of the solvent 0·00197 for each gram present in 100 c.c. of solution. This value is uninfluenced by temperature (10–30° C.) or the presence of acids, bases, and salts. I have obtained practically identical values for the haemocyanins of *Busycon*, *Limulus*, and *Homarus* as the data in Table V show. These results are of little theoretical interest aside from showing the great similarity of the various haemocyanins in this regard, and demonstrating their general resemblance to other proteins. They form the basis, however, of a very convenient method of estimating approximately the concentration of haemocyanin solutions in the course of experimental work.

Table V. *Refractive index of haemocyanin solutions.*

Species	Concentration of haemocyanin %	Temp. ° C.	Solvent	Refractive index of solution	Refractive index of solvent	$\frac{N-N_0}{c}$
*Octopus vulgaris**	1·134	—	0·05 NaOH	1·336166	1·333931	0·00197
	1·134	—	0·05 HCl	1·335938	1·333697	0·00198
	1·134	—	1 *N* NaCl	1·337088	1·334857	0·00197
Busycon canaliculatum	2·18	20	H_2O	1·33724	1·33300	0·00194
	10·97	24	H_2O	1·3543	1·3326	0·00198
	3·656	23	H_2O	1·34000	1·33279	0·00197
	1·083	23	H_2O	1·33494	1·33297	0·00198
Limulus polyphemus	4·90	15·2	H_2O	1·34303	1·33329	0·00198
	4·90	15·2	5 % KCl	1·34654	1·33682	0·00198
	7·26	23	H_2O	1·34715	1·33275	0·00198
Homarus americanus	11·85	20	H_2O	1·35668	1·33300	0·00200

* Selected from Quagliariello (1920*a*).

(2) *The absorption of light by haemocyanin.*

The earlier work on the absorption of light by haemocyanin was reviewed by Dhéré and Burdel (1919), and Dhéré, Baumeler and Schneider (1929) in papers in which they described original observations on the oxyhaemocyanin of *Helix*, *Sepia*, *Palinurus*, *Homarus*, and *Limulus* made with a spectrographic technique. Dhéré and Burdel pointed out that the apparent absorption of light by haemocyanin solutions is of a complex nature, and is due in part to colloidal phenomena, "réflexion lumineuse sur les grosses particules colloïdales," and in part to selective absorption by the molecule of oxyhaemocyanin. They found a band of maximal absorption in the visible range, the axis of which varied between 571 and 581 $m\mu$. They concluded that variation in the situation of this axis depends not only on the electrolytes present in the solvent but also on the species of animal which yielded the pigment.

The employment of spectrophotometric technique by Vlès (1913), Quagliariello (1922), Begemann (1924), Svedberg and Chirnoaga (1928), Svedberg and Heyroth (1929*a*), and Redfield (1930*a*) enables the absorption of light by haemocyanin solutions to be described more precisely.

(3) *The scattering of light.*

Redfield showed that the apparent absorption of light in the visible part of the spectrum by specimens of blood containing reduced haemocyanin was due solely to the Tyndall effect, that is, the scattering of light by the constituents of the solution. This was demonstrated by the fact that the extinction coefficients of the reduced blood at various wave-lengths vary with the reciprocal of the fourth power of the wave-length. This relation was deduced by Lord Rayleigh for the loss of intensity through scattering when light is passed through a medium con-

taining particles small when compared to the wave-length. The data in Fig. 3 demonstrate this relationship. The light emerging from the solution at an angle of 90° from the incident beam, when viewed through a Nicol prism, is nearly completely polarised, as required by the theory of scattering. This fact indicates that haemocyanin solutions are not fluorescent, as claimed by Lankester (1871) and Jolyet and Regnard (1877).

The amount of light scattered by the haemocyanin present in blood varies greatly in different species, being largest in the gastropod *Busycon*, least in the

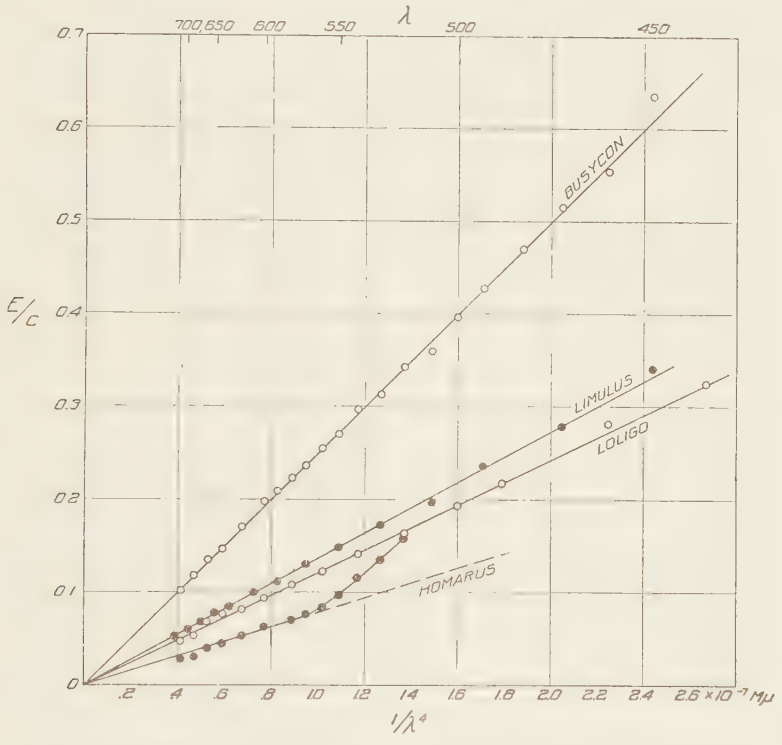

Fig. 3. Extinction coefficients, E/c, of reduced blood plotted against the reciprocal of the fourth power of the wave-length, $1/\lambda^4$.

squid and lobster. Purified preparations of reduced haemocyanins dissolved in water are almost water-clear and colourless and appear to scatter very little light, except at reactions in the neighbourhood of the isoelectric point. The scattering is greatly increased by the presence of electrolytes, and particularly by magnesium and calcium (Dhéré and Burdel, 1919; Redfield, 1930*a*; Redfield and Ingalls, 1932). Under these conditions, however, the scattering is diminished by the presence of alkali and, in the case of *Busycon* haemocyanin, the effect of the addition of sodium chloride disappears at reactions more alkaline than *p*H 8.

The interpretation of the phenomena of scattering is open to some doubt. Dhéré's suggestion that it is due to reflection of light from the large colloidal

particles is not strictly tenable, since such reflection does not vary with the reciprocal of the fourth power of the wave-length (Burton, 1921). The relation deduced by Rayleigh requires that the particles be *small* compared to the wave-length. Mecklenberg has shown that the relation holds for sulphur particles when the diameter of the particle falls between 5 and 93 $m\mu$. Since the radius of the molecules of the haemocyanins of *Helix* and *Limulus* are of the order of 10 $m\mu$, according to Svedberg and Heyroth (1929*a*), we may expect the Rayleigh equation to apply in their case. While it has frequently been assumed that the intensity of the Tyndall phenomenon depends upon the size of the particles, recent studies have indicated that the scattering of light from liquids is to be accounted for by local fluctuations in density due to thermal agitation, rather than by considerations of the size of the dissolved particles. From such considerations Raman (1927) has developed a theory of scattering by colloidal solutions in accordance with which it appears possible to relate the observed optical phenomena to the osmotic pressure of the solutions, as controlled by the equilibrium between electrolytes and protein. Until this possibility is examined quantitatively it is improper to draw inferences concerning the size of the particles of haemocyanin, or their degree of aggregation from the phenomenon of scattering.

(4) *The spectrum of the chromatic group.*

If it be assumed that the amount of scattering is the same in oxygenated as in reduced solutions of haemocyanin, it is possible to correct the absorption spectrum of the oxygenated solution for the scattering and thus obtain a measure of the absorption of light by the chromatic group formed by the union of oxygen with the portion of the molecule containing copper. Fig. 4 shows the absorption curves of oxygenated and reduced blood of *Limulus*. The intermediate curve, obtained by subtracting the ordinates of the curve for reduced blood from those of the oxygenated blood at each wave-length, represents the corrected spectrum of the chromatic group. By this method the haemocyanins of different species may be compared. Fig. 5 shows the spectrum of the chromatic groups of four species. These spectra display a considerable similarity, indicating a close chemical relationship. There exist, however, definite differences in the spectra of each species. The spectra of the chromatic group of a given species vary very little, if at all, as the result of alterations in hydrogen-ion concentration and salt content, and the differences in the spectra of different species persist after the process of purification.

The species compared by Redfield (1930*a*) represent widely separated groups of animals. Measurements of the absorption of light in the visible part of the spectrum by the blood pigments of the gastropods have shown that the haemocyanins of *Helix*, *Paludina*, and *Littorina* are distinctly different with regard to the chemical constitution of the active group of the molecule (Svedberg and Hedenius, 1933). In *Helix* the maximum absorption of light by the oxyhaemocyanin occurs at about 550 $m\mu$; in *Paludina* at 600 $m\mu$.

(5) *Absorption in the ultra-violet.*

Dhéré (1920) found that the haemocyanin of a number of molluscs and crustaceans showed two bands of absorption in the ultra-violet. The band with axis at 278$m\mu$ was attributed to the protein portion of the molecule, being common to other proteins such as serum albumen, egg albumen, and serum globulin. The band having an axis at 346$m\mu$ was considered by Dhéré to be a specific characteristic of haemocyanin and was attributed to the prosthetic group. He believed that this

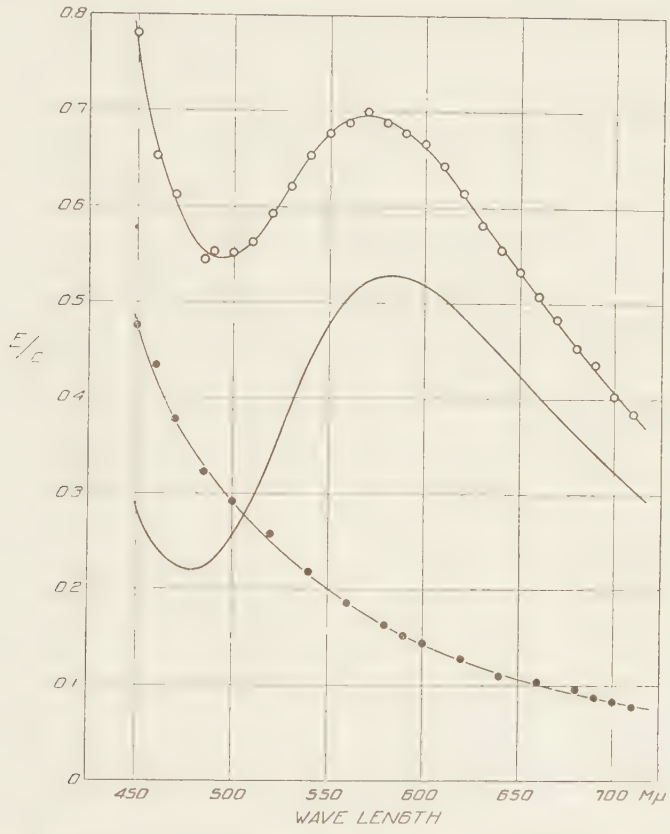

Fig. 4. Absorption spectra of blood of *Limulus polyphemus*. Upper curve, oxygenated blood; lower curve, reduced blood; intermediate curve, corrected spectrum of chromatic group.

group corresponded to the γ band of oxyhaemoglobin, which may be assigned to the porphyrin of the prosthetic group of this substance, since a band in the same region characterises the spectrum of haematoporphyrin. Roche and Dubouloz (1933) find that the band at 346$m\mu$ disappears when the haemocyanin is reduced, and is replaced by progressive absorption between 380 and 300$m\mu$. The ultra-violet absorption bands of oxyhaemocyanin are clearly demonstrated in the spectrophotometric curves published by Svedberg and Heyroth (1929*a*) for the haemocyanins of *Helix* and *Limulus*. The extinction coefficient for unit concentration is lower in *Limulus* than in *Helix*.

(6) *Beer's law.*

Quagliariello (1922) and Svedberg and Heyroth (1929*a*) present evidence that the extinction coefficient of haemocyanin solutions is not proportional to the concentration of haemocyanin. Redfield (1930*a*) showed that for solutions of haemocyanin of *Busycon* and *Limulus* Beer's law held accurately in the visible region for a range of concentrations from 0·2 to 5·5 per cent. The observations of Svedberg and Heyroth were made in the ultra-violet and for concentrations ranging

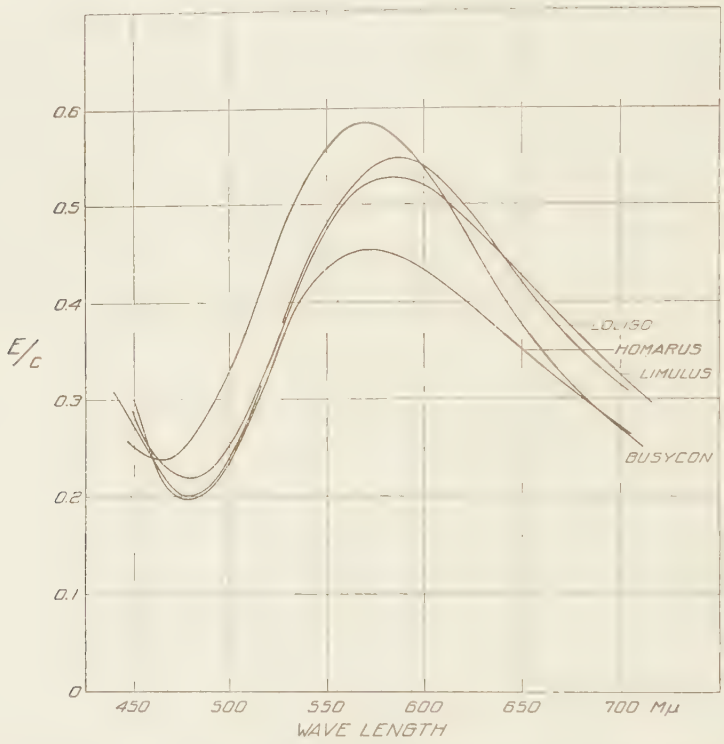

Fig. 5. Absorption spectra of chromatic groups of haemocyanin in blood of various species. Ordinate, E/c, measures extinction coefficient per milligram atom of copper per litre of blood.

from 0·136 to 0·030 per cent. The change in the light absorption for unit concentration was associated with alterations in the size of the protein units. It seems probable that the failure of Beer's law under these circumstances is due to alterations in the scattering effect, rather than to changes in true light absorption.

IV. THE PROPERTIES OF THE PROSTHETIC GROUP.

The properties of haemocyanin which have been reviewed so far result for the most part from the fact that the haemocyanins are proteins. From the physiological point of view these properties are not without interest. As globulins their characteristic solubility in salt solutions fits them for circulation in the body fluids; as

bodies of large molecular size they are readily retained within the walls of the fluid spaces in which they circulate; as ampholytes they serve as buffers in the blood, take part in the transport of carbon dioxide, and influence the equilibrium of electrolytes across the membranes bathed by the blood (Thomas, 1929). These properties are not uniquely characteristic of the haemocyanins, however, being shared by a large number of other proteins. It is the prosthetic group which enables haemocyanin to unite with oxygen and gives it its significance in respiration.

The copper complex isolated by Conant and Humphrey (1930) in their study of the prosthetic group of *Limulus* haemocyanin was insoluble in aqueous solutions. It tended to form soluble complexes with amines and denatured albumen. They suggest that the function of the prosthetic group, like that of protoporphyrin in haemoglobin, is to provide a basis for a stable metallic complex, capable of combining with oxygen. By its union with protein, this complex is rendered soluble in the blood.

(1) *Oxygenation.*

Dhéré (1900, 1903, 1915) was the first to examine the proportionality between the amount of copper present in haemocyanin solutions and the quantity of oxygen with which they would combine. It is now well established through the investigations of Begemann (1924), Redfield, Coolidge and Montgomery (1928), Guillemet and Gosselin (1932), who have examined the haemocyanin from thirteen species of animals, that one molecule of oxygen is combined with a quantity of haemocyanin containing exactly two atoms of copper. This ratio differs from that characterising haemoglobin, in which one molecule of oxygen corresponds to one atom of iron, and haemerythrin in which one molecule of oxygen appears to correspond to three atoms of iron (Florkin, 1933).

(2) *The equilibrium of haemocyanin with oxygen.*

The equilibrium between haemocyanin and oxygen has been studied in some detail by Begemann (1924), Pantin and Hogben (1925), Wolvekamp (1932), and by Hogben, Stedman, and the author and their collaborators. The oxygen dissociation curves of bloods containing haemocyanin are similar in a general way to those of bloods containing haemoglobin, and as a first approximation many of these curves can be described by the equation proposed by Hill to express the equilibrium between haemoglobin and oxygen (Hogben, 1926; Stedman and Stedman, 1926*a*; Redfield, Coolidge and Hurd, 1926). As in the case of haemoglobin, the equilibrium is influenced by hydrogen-ion activity; but between haemoglobin and haemocyanin and between the various species of haemocyanin there are important quantitative differences in this regard. Speaking generally, it may be stated that in the case of the Crustacea (*Homarus*, *Maia*, *Palinurus*, *Cancer*) and the cephalopod, *Loligo*, increasing acidity decreases the stability of oxyhaemocyanin. This effect is analogous to that first observed by Bohr, Hasselbach, and Krogh in the case of haemoglobin. The magnitude of the effect is much greater, however, for at acid reactions (pH 6) several hundred millimetres of oxygen

pressure may be necessary to produce half-saturation of the solution with oxygen (Fig. 6). At greater acidities also the stability of oxyhaemocyanin increases, and the "Bohr effect" is reversed, at least in the case of the Crustacea (Fig. 7). The haemocyanins which occur in the blood of *Limulus* and of the gastropods, *Helix* and *Busycon*, on the other hand, never require high oxygen pressures to produce

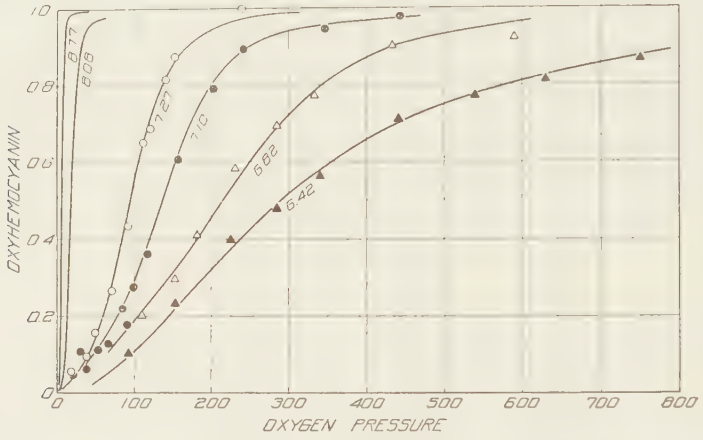

Fig. 6. Oxygen dissociation curves of blood of the lobster, *Homarus americanus*, at alkaline reactions.

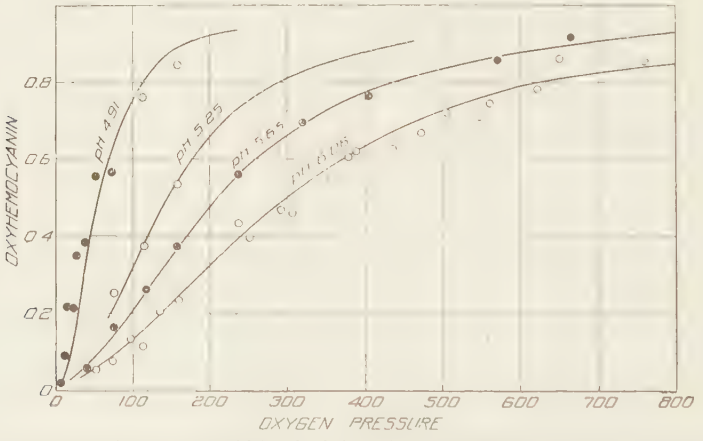

Fig. 7. Oxygen dissociation curves of blood of the lobster, *Homarus americanus*, at acid reactions.

half-saturation. In the physiological range of hydrogen-ion activities the Bohr effect is reversed, the stability of the oxyhaemocyanin increasing as the solutions become more acid (Fig. 8). At more alkaline reactions (pH 8·3 and beyond) the stability also increases so that under these conditions the Bohr effect is like that displayed by haemoglobin (Fig. 9).

The oxygen equilibrium of purified haemocyanin. In the case of the haemocyanin of *Helix*, purified by dialysis (Stedman and Stedman, 1928), and that of *Limulus* and *Busycon*, purified by precipitation at the isoelectric point (Redfield, 1930*b*;

Redfield and Ingalls, 1932), the equilibrium with oxygen may be described by the mass law, on the assumption that the various oxygen-combining groups react independently of one another in their combination with oxygen. The reaction may thus be written:

$$Hcy + O_2 = Hcy\,O_2 \quad \text{or} \quad K = \frac{(Hcy\,O_2)}{(Hcy)(O_2)}.$$

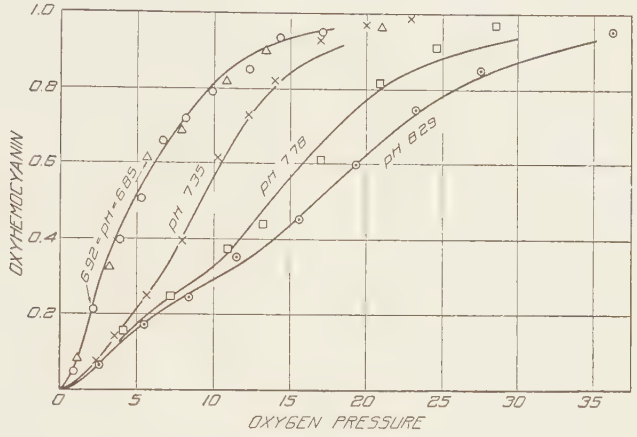

Fig. 8. Oxygen dissociation curves of blood of *Limulus polyphemus* between *p*H 6·85 and 8·29.

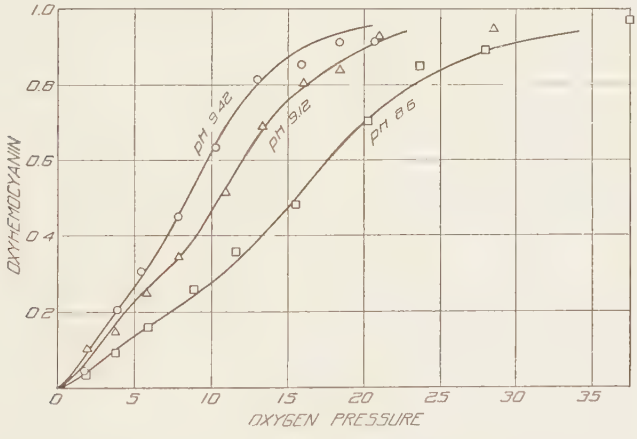

Fig. 9. Oxygen dissociation curves of blood of *Limulus polyphemus* between *p*H 8·6 and 9·42.

Fig. 10 illustrates this relation in the case of *Limulus* haemocyanin, the curve being drawn through the points in accordance with the above equation. This finding is similar to that made by Barcroft and Roberts (1909) in the case of purified haemoglobin, an observation which subsequent workers have found difficult to confirm. It is of interest in showing that the mass law may be applied to the oxygenation of respiratory proteins and in indicating that the more complicated dissociation curves displayed by haemocyanin in native blood are to be explained by some

modification of the mass law. The simplicity of the relationship favours the use of these materials in studying the effect of various factors on the equilibrium.

Stedman and Stedman (1928) detected no change in the value of the oxygen dissociation constant of *Helix* haemocyanin at *p*H values ranging from 4·04 to 9·02. The equilibrium constant of *Limulus* haemocyanin decreases as the *p*H value increases from 4·5 to 10·4 (Redfield, 1930*b*). A recalculation of the Stedmans' data suggested that a small change in the value of the oxygen equilibrium constant may occur in the case of *Helix* also.

The oxygen dissociation constant of purified *Busycon* haemocyanin was found to change quite abruptly between *p*H 8 and 9 (Redfield and Ingalls, 1932). This result was explained on the assumption that at this reaction a salt is formed by some acid- or base-binding group of the protein, and that this salt has an affinity for oxygen different from that of the unneutralised substance.

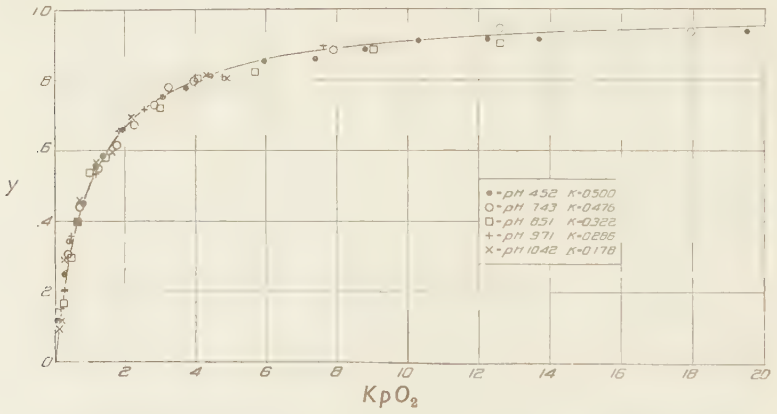

Fig. 10. Oxygen dissociation curve of purified haemocyanin of *Limulus polyphemus*. Ordinate, *y*, the fraction of haemocyanin present as oxyhaemocyanin. Abscissa is the product of the oxygen pressure, pO_2, and the oxygen equilibrium constant, *K*. The curve is drawn in accordance with the mass law.

The effect of adding electrolytes to purified haemocyanin solutions was examined by Hogben and Pinhey (1926, 1927). While these experiments did not lead to definite conclusions, they indicated that the presence of electrolytes might alter the affinity of haemocyanin for oxygen. In the case of *Busycon* haemocyanin Redfield and Ingalls found that the form of the oxygen dissociation curve was unchanged by the addition of sodium, potassium, or magnesium chlorides, or potassium phosphate to an ionic strength of 0·5. Calcium chloride appeared to alter the shape of the oxygen dissociation curve[1].

The addition of 0·5*M* NaCl to purified *Busycon* haemocyanin solutions does not change the value of the oxygen equilibrium constant at acid or the more alkaline reactions. It does, however, lower the *p*H value at which the oxygen

[1] I have also found that the addition of sea water to purified *Limulus* haemocyanin causes the curve to become distinctly sigmoid in shape. The curve does not coincide with that of the haemocyanin in native blood at the same hydrogen-ion concentration.

equilibrium constant changes by about one *p*H unit. It appears that electrolytes alter the affinity of haemocyanin for oxygen principally by their effect on the dissociation constant of an acidic or basic group associated with the prosthetic group rather than directly through an effect upon the oxygen equilibrium constant.

The effect of temperature upon the equilibrium of haemocyanin with oxygen has been studied in the case of a number of bloods, both in the native and dialysed condition, by Hogben (1926), Hogben and Pinhey (1926, 1927), and Redfield and Ingalls (1933). In all cases the affinity of haemocyanin for oxygen decreases with increasing temperature. The oxygenation of haemocyanin, like haemoglobin, may be considered to be an exothermic reaction. In these cases the form of the oxygen dissociation curve is so complicated that one cannot estimate the heat of reaction without making complicating assumptions to explain the form of the curve itself. In the case of the purified haemocyanins of *Limulus* and *Busycon*, which yield dissociation curves in conformity to the mass law, the oxygen equilibrium constant is arrived at simply and the effect of temperature on its value may be easily interpreted. Brown (1933) has shown that the heat evolved in the oxygenation of these haemocyanins is approximately 14,800 calories per gram-molecule of oxygen for *Limulus* haemocyanin and 16,000 calories for *Busycon* haemocyanin.

The oxygen equilibrium of haemocyanin in native bloods. The oxygen dissociation curves of haemocyanin as it exists in the blood, and also those of the dialysed haemocyanin of the Crustacea (Stedman and Stedman, 1926*a*, *b*), do not have the simple hyperbolic form required by the mass law, if it is supposed that the oxygen-binding groups of the haemocyanin are situated upon separate molecules or are so separated in position on a common molecule that their reactions are independent of one another. The departure of these curves from the hyperbolic form indicates some sort of interdependence of associated prosthetic groups.

In the case of haemoglobin this interdependence has been explained by the assumption that the haemoglobin molecule consisted of aggregates of several oxygen-binding units so constituted that the oxygenated form was only stable when all of the associated groups were combined with oxygen (Hill, 1910) or that the oxygenation of the four prosthetic groups of the haemoglobin molecule takes place as a series of steps, each characterised by a distinct oxygen dissociation constant (Adair, 1925*a*, *b*).

By a development of the former view it is possible to account for the form of all of the oxygen dissociation curves of bloods containing haemocyanin which have been accurately described (Redfield and Ingalls, 1933). Under certain conditions of temperature and hydrogen-ion activity, the oxygen dissociation curves of the lobster and the squid are precisely described by the equation developed by Hill on the assumption that the prosthetic groups are associated in groups of two; under other conditions the data are fitted by curves calculated on the assumption that they are associated in groups of four. According to Hill's theory the fraction of the respiratory protein in the oxygenated condition, y, at any oxygen pressure, x, is given by the expression:

$$y = \frac{Kx^n}{1 + Kx^n},$$

where K is the oxygen equilibrium constant and n is the number of prosthetic groups so associated as to form a stable oxygenation product. There is thus evidence that haemocyanin may exist in forms characterised by values of $n = 1$, 2, and 4.

The haemocyanins in the blood of the squid and lobster, under conditions intermediate between those under which n equals 2 or 4, yield oxygen dissociation curves which cannot be fitted by Hill's equation using any constant value of n. This is true under practically all conditions of the haemocyanins of the blood of *Limulus* and *Busycon*, the curves of which may have a distinctly undulatory character (Figs. 8, 9, 11). These curves may be described, however, by assuming the simultaneous presence of two or more forms of haemocyanin, each characterised by a different integral value of n. The most general form of the expression defining this assumption is:

$$y = \frac{\alpha_1 K_1 x^1}{1 + K_1 x^1} + \frac{\alpha_2 K_2 x^2}{1 + K_2 x^2} + \frac{\alpha_3 K_3 x^3}{1 + K_3 x^3} + \frac{\alpha_4 K_4 x^4}{1 + K_4 x^4}, \text{ etc.,}$$

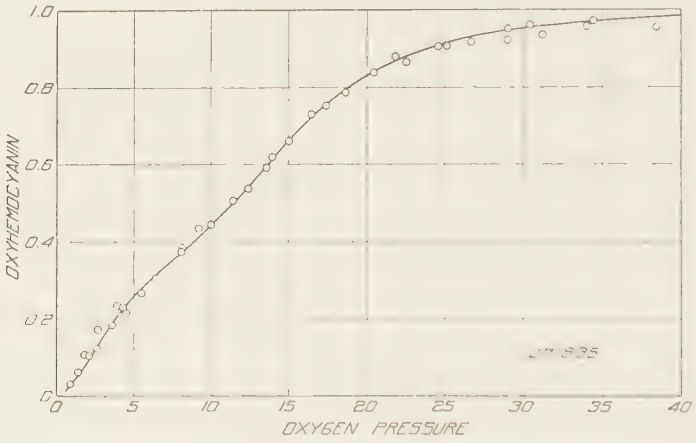

Fig. 11. Oxygen dissociation curve of haemocyanin in blood of *Busycon canaliculatum*.

where K_1, K_2, K_3, K_4, etc., are the oxygen equilibrium constants of the forms of haemocyanin characterised by values of $n = 1$, 2, 3, 4, etc., and α_1, α_2, α_3, α_4, etc., are the fraction of the total oxygen bound by each of these forms.

In most cases it is not necessary to assume the simultaneous presence of more than two of the forms described by each term of the equation and practically it is never necessary to postulate the presence of a form characterised by values of n equal to three or greater than four, so that the expression used in analysing the curves may be simplified and does not require the use of terms which cannot be shown to apply alone under appropriate conditions to certain haemocyanins.

When the oxygen dissociation curves of various haemocyanins are examined systematically under varying conditions of hydrogen-ion activity and temperature and analysed in accordance with the above equation, it is found that the constants resulting from the analysis vary in a systematic manner. The values of α_1, α_2, and α_4 indicate that in passing from acid to alkaline reactions the state of the haemocyanin

changes from the form characterised by a low value of n to one characterised by a higher value of n. At more alkaline reactions (pH 8·3 or greater) a reverse change

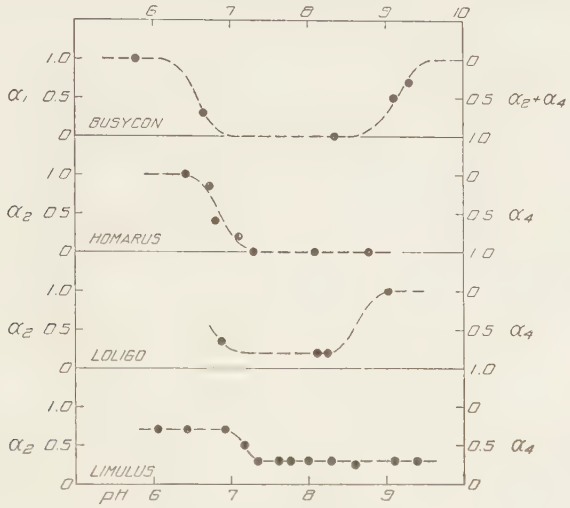

Fig. 12. Variation with pH of values of α characterising the haemocyanin of different species.

occurs in the case of the bloods of *Busycon* and *Loligo*. The values of the oxygen equilibrium constants K_1, K_2, and K_4 tend to decrease, indicating decreased stability

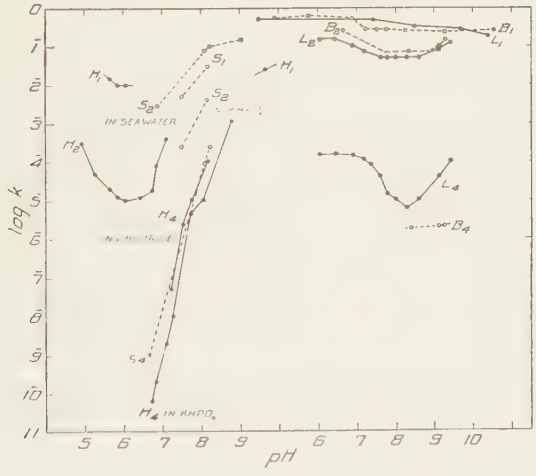

Fig. 13. Variation with pH of values of K characterising the haemocyanin of different species. H_1, H_2, H_4 indicate values of K_1, K_2, K_4 for *Homarus* blood. S_1, S_2, S_4 indicate values of K_1, K_2, K_4 for squid blood. B_1, B_2, B_4 indicate values for *Busycon* blood. L_1, L_2, L_4 indicate values for *Limulus* blood.

of the oxyhaemocyanin with decreasing acidity. After passing through a minimum, at a reaction characteristic of each species, the oxygen equilibrium constants increase again. These changes in the values of α and K, characterising various species, are summarised in Figs. 12 and 13.

It is noteworthy that the oxygen equilibrium constants characterising the haemocyanins of the squid and the lobster, on the one hand, are very similar in magnitude and in the way they vary with *p*H, and that those of *Limulus* and *Busycon*, on the other hand, have a similar resemblance, though differing markedly from the former species. The similarities and differences in the two groups suggest that some difference in structure of the prosthetic group of the haemocyanins of the squid and the lobster distinguishes these proteins from the haemocyanins of *Busycon* and *Limulus*.

Increasing temperature appears to change the haemocyanins of *Limulus* and the lobster from forms characterised by low values of n to forms characterised by higher values of n. The various oxygen equilibrium constants, K_1, K_2, and K_4 decrease in value with rising temperature as required if the oxygenation is an exothermic reaction.

(3) *The kinetics of oxygenation.*

G. Millikan (1933) has studied the rate at which haemocyanin becomes oxygenated or reduced, employing a continuous flow apparatus similar in principle to that used by Hartridge and Roughton in studying the oxygenation of haemoglobin. He finds that these reactions proceed with great rapidity. *Limulus* serum is dissociated from half its oxygen in 0·1 sec., and the serum of the crab, *Maia squinado*, in about 0·04 sec. These haemocyanins become half-saturated with oxygen in less than 0·003 sec. These velocities correspond in order to those observed for haemoglobin by Hartridge and Roughton (1925). Clearly these reactions are too rapid to limit the animals' activity appreciably.

The kinetic experiments provide independent support for the application of the theory of mass action to the oxygenation of haemocyanin. The rates of oxygen combination and oxygen dissociation were measured separately for dialysed *Limulus* haemocyanin. From these measurements the equilibrium constant for the haemocyanin was calculated and found to agree with that obtained by Redfield (1930*b*) from equilibrium studies.

Different haemocyanins were observed to differ greatly both in the speed of their reaction with oxygen and also in the way these speeds are affected by changes in hydrogen-ion activity. The haemocyanin of *Limulus* serum shows relatively little change in the rate of oxygen dissociation on altering the *p*H, while that of *Maia* increases its rate of oxygen dissociation tenfold as the acidity is increased from *p*H 9 to *p*H 4.

(4) *Methaemocyanin.*

The union of oxygen with haemocyanin is not an oxidation in the electronic sense, but is analogous to the change of haemoglobin to oxyhaemoglobin to which Conant (1923) has applied the term *oxygenation*. The copper present in both the reduced and oxygenated haemocyanin is in the cuprous state. It may be oxidised by sufficiently strong oxidising reagents, such as molybdicyanide or permanganate, forming compounds analogous to methaemoglobin which may be called methaemo-

cyanins. According to Conant, Chow and Schoenbach (1933), the oxidation-reduction potential of the system *Limulus* haemocyanin-methaemocyanin is approximately +0·55 volt. The change involves one hydrogen equivalent for each atom of copper in the haemocyanin. Reiss and Vellinger (1929) have measured the electrode potentials of the haemocyanin of *Palinurus* as it is oxidised, and find the reaction is half complete at a potential of +0·13 volt.

Conant, Chow and Schoenbach find that, unlike methaemoglobin, methaemocyanin retains the power of combining oxygen reversibly (oxygenation) so that oxymethaemocyanins may be formed. It is for this reason that ferricyanide cannot be used to drive off the oxygen from oxyhaemocyanin in analytical procedures, as Cook (1928) observed, and accounts in part for the fact that early attempts to demonstrate the formation of methaemocyanin have been unsuccessful (Quagliariello, 1922).

(5) *The reaction of the prosthetic group with substances other than oxygen.*

Carbon monoxide. Craifaleanu (1919*b*) observed that when carbon monoxide is bubbled through haemocyanin solutions they become colourless. Since such solutions recolour more slowly when again exposed to oxygen than is the case if the haemocyanin is reduced with an inert gas, he concluded that probably a carbon monoxide-haemocyanin compound was formed which is unstable in the presence of oxygen. Dhéré and Schneider (1922*a*) could see no difference between the action of carbon monoxide and the inert gases such as nitrogen; however, they hesitated to conclude that carbon monoxide formed no compound with haemocyanin. They pointed out that if such a compound were formed it must be colourless and less stable than oxyhaemocyanin.

In recent experiments Root (1934) has shown that carbon monoxide combines with the haemocyanin of *Limulus* in proportions similar to those of oxygen. The compound is indistinguishable from reduced haemocyanin in colour. The affinity of haemocyanin for carbon monoxide is only one-twentieth as great as for oxygen.

Hydrocarbons. Dhéré and Schneider (1922*b*) find no evidence that the haemocyanin of *Helix* forms coloured compounds with methane, ethylene, or acetylene as claimed by Griffiths.

Nitrogen dioxide. Dhéré and Schneider (1919, 1920, 1922*b*) have shown that the haemocyanins of *Helix*, *Octopus*, and *Eledone* form a green compound on treatment with NO_2 which has been isolated in crystalline form. The nitrogen dioxide-haemocyanin is much more stable than oxyhaemocyanin. The haemocyanin of *Astacus* appears to form this compound with difficulty and that of the lobster not at all.

Cyanide. Kobert (1903) observed that haemocyanin treated with KCN becomes colourless. Craifaleanu (1919*b*) pointed out the analogy of this phenomenon to the behaviour of other complex copper salts, such as the green albuminate of copper and copper ammonium salts which are likewise decolorised by the addition of cyanide. The cyanide forms a compound with haemocyanin called cyanhaemo-

cyanin. This compound does not combine with oxygen (Cook, 1928). The reaction forms the basis for methods of estimating the oxygen combined with haemocyanin by means of the Van Slyke analysis (Redfield, Coolidge and Montgomery, 1928; Stedman and Stedman, 1926*b*). Craifaleanu found that solutions of *Octopus* cyanhaemocyanin which did not contain an excess of cyanide became blue again if exposed to oxygen, and concluded that cyanhaemocyanin is an unstable compound tending to change into oxyhaemocyanin in the presence of oxygen. Experiments made by Dr Izquierdo and Mr Pearson in the author's laboratory have failed to demonstrate any simple stoichiometric relation between the quantity of cyanide present and the loss of colour or oxygen capacity by the solutions. The results suggest that the equilibrium between cyanide and haemocyanin is a reversible one such as that represented by the following equation, in which $R\mathrm{Cu}_2$ represents a quantity of haemocyanin combining with one molecule of oxygen:

$$R\mathrm{Cu}_2 + 4\mathrm{NaCN} \rightleftharpoons R\mathrm{Cu}_2(\mathrm{CN})_4.$$

Hydrogen sulphide. Craifaleanu (1919*b*) found that hydrogen sulphide decomposes haemocyanin. A portion of the protein is precipitated and copper is set free and precipitated as copper sulphide.

Acids and bases. The large number of acid- and base-binding groups present in the protein portion of the haemocyanin molecule make it difficult to demonstrate that the prosthetic group has acidic or basic qualities. There is, however, some indirect evidence that this is the case.

Carbon dioxide behaves like the inert gases in reducing oxyhaemocyanin (Dhéré and Schneider, 1922*a*). If it forms a compound with the prosthetic group, this compound is colourless. The presence of carbon dioxide and other acids, which alter the hydrogen-ion concentration of the solutions, influences the affinity of haemocyanin for oxygen, and as in the case of haemoglobin, it has been assumed that this action is due to the formation of salts by acid- or base-binding groups composing a part of the prosthetic group or closely associated with it (Redfield and Ingalls, 1932, 1933). The fact that the form of the oxygen dissociation curve of certain haemocyanins varies with the salts in which the haemocyanin is dissolved may be due to a reaction between the ions of the solvent and the prosthetic group of the protein.

A particularly interesting case in this connection is the effect of hydrochloric acid on the purified haemocyanin of *Limulus*. The haemocyanin reacts with hydrochloric acid to form a colourless compound, unable to combine with oxygen. This compound may be separated from a partially acidified haemocyanin solution by the addition of a strong solution of sodium chloride. The equilibrium between hydrochloric acid, haemocyanin, and the resulting colourless component may be described by an equation derived on the assumption that the latter is a salt formed as the result of a reaction in which the haemocyanin behaves as a divalent acid or base. The reaction is half complete at *p*H 3·7 and is complete upon the addition of approximately 110×10^{-5} mols HCl per gram of haemocyanin. From comparison with the acid titration curve of haemocyanin, which is half complete at *p*H 3·3 and

requires 160×10^{-5} mol per gram for completion, Redfield, Mason and Ingalls (1932) concluded that this reaction involves only a limited portion of the total basic groups, perhaps only those in the radical immediately associated with oxygen transport.

V. SPECIFICITY AND NOMENCLATURE.

There can be no doubt that the haemocyanins of the four principal groups, cephalopods, gastropods, Crustacea, and Xiphosura, are distinctly different in their chemical properties. To what extent these proteins are the same in related species is as yet uncertain, but it seems clear that smaller differences separate the haemocyanins of species belonging to the same class (Stedman and Stedman, 1926*a*, *b*, 1927; Hogben, 1926; Svedberg and Hedenius, 1933). As in the case of haemoglobin, the most clear-cut differences seem to be attributable to the properties of the protein portion of the molecule. Knowledge of the chemical structure of the prosthetic group of haemocyanin is still too limited to justify any statement regarding its similarity or difference in different groups of animals, though there can be no doubt that important differences do characterise its function in binding oxygen in different species. As pointed out above, there is some evidence that the haemocyanins may be divided into two groups having oxygen equilibrium constants varying in a characteristic way with hydrogen-ion activity, and these characteristics may depend on minor differences in the structure of the prosthetic group.

There is a tendency among workers in natural products to apply new names to any substance which can be shown to have any degree of individuality. In the case of such substances as haemocyanin, which appear to be characterised by different properties in almost every species examined, the nomenclature is apt to become needlessly complicated unless based on thorough chemical knowledge. For this reason I have disregarded the terms *haemosycotypin* proposed by Mendel and Bradley for the haemocyanin of *Busycon*, and *l*-, *o*-, and *h-haemocyanin* employed by Svedberg and his collaborators for the haemocyanins of *Limulus*, *Octopus*, and *Helix*. Until the various haemocyanins can be given a rational chemical classification, it seems less likely to lead to confusion if the term "haemocyanin" be reserved for all proteins having a copper complex as a prosthetic group, and if the haemocyanins of the various animals be distinguished by the scientific name of the species in which they occur.

VI. PHYSIOLOGICAL CONSIDERATIONS.

The physiological function of haemocyanin was definitely established in the case of the cephalopods by Bert (1867), who observed the change in colour of the blood, indicative of the oxygenation of the haemocyanin, as the blood passed through the gills. Analyses of the oxygen and carbon-dioxide content of the arterial and venous blood of the octopus (Winterstein, 1909) and squid (Redfield and Goodkind, 1929) demonstrate the magnitude of the change in these constituents and indicate that a very large proportion of the oxygen combined with the haemocyanin is utilised at each circuit of the circulation. Comparable observations do

not appear to have been made on other groups of animals, in which the circulatory apparatus is less conveniently arranged for physiological observation.

Certain inferences of physiological interest may be drawn from the chemical properties of the blood. The concentration of haemocyanin in the blood, and the amount of oxygen bound per gram of haemocyanin determine the total amount of oxygen which the blood may carry. The measurements of the oxygen capacity of various bloods recorded in Table VI show that in no case is the oxygen capacity of the blood as great as that obtaining in many animals possessing haemoglobin. Presumably the fact that haemocyanin occurs in solution rather than corpuscles limits, through considerations involving solubility and viscosity, the concentration of haemocyanin which may be circulated effectively.

Table VI shows that the copper content of the blood of various animals varies between about 2–20 mg. per 100 c.c. Sea water contains approximately 10 mg. of copper per cubic metre (Atkins, 1932). The copper in the blood represents a concentration over that of sea water of some 10,000-fold. Löhner (1924) has found that the fresh-water pulmonates, *Physa fontinalis* and *Lymnaea peregra*, which he considers to possess haemocyanin, are sensitive to poisoning by minute amounts of copper just as are other animals which are not provided with respiratory proteins which contain copper.

The bloods having the highest oxygen capacity are found in the cephalopods, which are also the most active of the large marine invertebrates. A similar correlation may be drawn between the activity of the animals and the pressures of oxygen required to saturate the blood. The bloods of *Limulus* and the gastropods are saturated at low oxygen pressures, as judged from the dissociation curves, and thus fit these animals for life under poor conditions of aeration, but limit the activity by maintaining the oxygen pressure of the blood at a low level during the period when oxygen is being given up to the tissues. In contrast the blood of the squid is oxygenated only at relatively high pressures and as a result the squid is extremely sensitive to oxygen lack, but is capable of superb activity when the conditions are favourable for the oxygenation of the blood. The crustaceans occupy a somewhat intermediate position. While their haemocyanin resembles that of the squid in many ways, its concentration is too low to provide a very adequate accessory to violent activity. The high oxygen pressures required to saturate the haemocyanin, especially in the presence of carbon dioxide or other acids, makes animals of this class very sensitive to asphyxiation.

The Bohr effect has received much attention in connection with the respiration of vertebrates. It is recognised that the effect of carbon dioxide in turning out oxygen from the blood in the tissues and the reciprocal effect in the lungs are responsible for a significant part of the mammalian respiratory exchange. In the case of the squid, in which the Bohr effect is large, it is estimated that one-third of the respiratory exchange results from this phenomenon (Redfield and Goodkind, 1929). The conditions leading to asphyxiation are determined by the properties of the oxygen dissociation curve as influenced by carbon dioxide, death occurring when the oxygen and carbon-dioxide pressures are such that the arterial blood can

Table VI. *Oxygen capacity, copper content, and haemocyanin content of blood of various animals. The oxygen capacity represents the amount of oxygen present when the blood is equilibrated with air. The copper content is calculated from this value assuming* 0·49 *volume per cent. to be dissolved and two atoms of copper present per molecule of oxygen combined. The haemocyanin concentration is calculated from the composition shown in Table I for the same species.*

Species	Oxygen capacity vol. %	Copper mg. per 100 c.c.	Haemocyanin %	Authority
Cephalopods				
Octopus vulgaris	3·1–4·5	1·48–22·8	5·9–9·1	Henze, 1901; Dhéré, 1903; Winterstein, 1909; Craifaleanu, 1919*a*
Loligo pealei	3·8–4·5	18·8–22·8	7·2–8·8	Redfield, Coolidge and Hurd, 1926
Gastropods				
Helix pomatia	1·15–2·2	3·75–9·70	1·47–3·9	Cuénot, 1891; Dhéré, 1900; Begemann, 1924
Helix aspersa	1·2	4·03	—	Cuénot, 1900–1
Busycon canaliculatum	2·1–3·35	9·16–16·2	3·7–6·6	Redfield, Coolidge and Hurd, 1926
B. carica	1·36	4·94	—	Redfield, Coolidge and Montgomery, 1928
Crustacea				
Astacus fluviatilis	2·4	10·8	—	Dhéré, 1900
Homarus vulgaris	3·1	—	—	Dhéré, 1900
H. vulgaris	1·22	4·14–14·8	—	Stedman and Stedman, 1925
H. americanus	1·95	8·3	4·4	Redfield, Coolidge and Montgomery, 1928
Palinurus vulgaris	1·43–1·80	5·34–7·45	—	Winterstein, 1909; Stedman and Stedman, 1925
Cancer pagurus	1·6–2·3	6·31–10·3	—	Dhéré, 1903; Stedman and Stedman, 1925
C. irroratus	1·23–1·69	4·2–6·82	—	Redfield, Coolidge and Hurd, 1926
C. borealis	1·40	5·16	—	Redfield, Coolidge and Montgomery, 1928
Carcinus maenas	1·14–1·16	3·69–3·80	—	Begemann, 1924
Callinectes sapidus	1·29	4·54	—	Redfield, Coolidge and Hurd, 1926
Ovalipes ocellatus	1·78	7·33	—	Redfield, Coolidge and Montgomery, 1928
Maia squinado	0·84–1·75	1·99–7·16	—	Winterstein, 1909; Stedman and Stedman, 1925
Xiphosura				
Limulus polyphemus	0·74–2·7	1·42–12·6	0·8–7·3	Jolyet, 1895; Redfield, Coolidge and Hurd, 1926

combine only 0·5 to 1·5 volumes per cent. oxygen. Recent experiments by Dr Eric Fries indicate that the oxygen pressures at which asphyxiation occurs at various temperatures may also be correlated with the effect of temperature on the oxygen equilibrium in the blood.

In the gastropods and *Limulus* the Bohr effect is reversed under physiological conditions. It may be argued that this property of the blood favours the absorption of oxygen at the gill. The ease with which the phenomenon may be shown to be

an advantage, irrespective of which way the system works, makes one very sceptical of the teleological argument.

The buffering of bloods containing haemocyanin does not appear to show any unique phenomena. Buffer action results from the protein nature of the respiratory pigments and is in a general way dependent on the concentration of these substances. Bloods containing haemocyanin are not inferior to other bloods of like oxygen capacity in buffer action, and inasmuch as buffering under normal conditions is concerned with the transport of carbon dioxide, there is no need for caring for an amount of carbon dioxide greater than that equivalent to the oxygen transport. Gram for gram haemocyanin is not an inferior buffer to haemoglobin, as the values cited on p. 187 show. It has been pointed out, in discussing the effect of oxygenation upon the titration curve of haemocyanin, that the change in the dissociation constant of the acid-combining group of haemoglobin which is effected on oxygenation falls within the series showing the magnitude of this phenomenon among the haemocyanins.

In conclusion it may be pointed out that while the quantitative differences which distinguish the various haemocyanins have made the comparative study of these compounds fertile from a chemical point of view, the bloods containing haemocyanin do not form as interesting an evolutionary series as do those provided with haemoglobin (Redfield, 1933). This is perhaps due to the fact that evolution among the haemocyanin-bearers has resulted in a great variety of forms, but has led to little advance in the mechanisms for respiration, whereas the series of animals provided with haemoglobin has culminated in the production of animals capable of intense activity in a terrestrial and aerial environment.

VII. SUMMARY.

The haemocyanins are proteins combined with a prosthetic group consisting of a complex copper salt of a sulphur compound and a polypeptide. The physical and chemical properties of haemocyanin are largely due to its protein nature, and the specific differences between the haemocyanins of different groups of animals result largely from the differences in the protein part of the molecule. The combination of haemocyanin with oxygen and the characteristic change in the absorption spectrum which results are, on the other hand, properties of the prosthetic group. The haemocyanin combines with oxygen in all cases in proportion to its copper content, one molecule of oxygen being combined by a quantity of haemocyanin containing two atoms of copper. There is some evidence that at least two modifications of the prosthetic group may occur characterising the Crustacea and cephalopods on the one hand, and the gastropods and *Limulus* on the other. The equilibrium with oxygen may be described by the mass law if it be assumed that under different conditions one, two, or four prosthetic groups must be oxygenated simultaneously in order to form a stable compound and that under certain conditions a mixture of such forms of the protein exist. In general the oxygenation phenomena displayed by haemocyanin differ from those of haemoglobin only in a quantitative sense.

The copper of the prosthetic group is in the cuprous condition but may be oxidised, forming methaemocyanin, by strong agents. Methaemocyanin will combine reversibly with oxygen, forming oxymethaemocyanin. Haemocyanin also combines with carbon monoxide, nitrogen dioxide and with cyanide, forming stable compounds.

The physiological function of haemocyanin as a respiratory pigment is well established in the case of the cephalopods. Some correlation exists between the activity of various animals, the oxygen capacity of the blood, and the pressures of oxygen under which the haemocyanin becomes oxygenated.

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THE RÔLE OF BACTERIA IN THE CYCLE OF LIFE IN THE SEA[1]

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The sea harbors an extensive population of bacteria, varying greatly in numbers and in the variety of their activities. This variation depends, in the case of the sea-water, on the distance from shore, depth of water, season of year, abundance of plankton and uniformity of mixing of the water, and in the case of the sea bottom, upon the nature of the bottom material, depth from the surface of the sediment, etc. These bacteria take part in a number of processes which are of considerable importance in the life of the plant and animal populations of the sea, namely, in the decomposition of plant and animal residues, in the transformation of their nitrogen to such an available form as ammonia and of their carbon to carbon dioxide, in the formation of nitrate as well as the reduction of nitrate, in the oxidation of sulfur and the reduction of sulfate, in the fixation of nitrogen, in the direct and indirect precipitation of calcium and iron, in the formation of organic matter or humus in the sea bottom and in the decomposition of this humus. To what extent these processes are absolutely essential in the growth of plant and animal life in the sea still remains to be determined.

The study of bacteria in the sea includes a series of problems in bacteriology and in biochemistry which are to be coordinated with other branches of oceanography, in order to establish the rôle of the bacteria in the marine processes. In most instances information on marine bacteriology has been gathered by individual investigators during short periods, using material obtained from few localities; less often the work is the result of an expedition, where limited facilities were offered for a detailed study of bacterial life and of bacterial processes under the natural conditions prevailing in the sea. Most of these investigations had a specific purpose of demonstrating either the presence or absence of certain bacteria in the sea, or of organisms capable of bringing about processes which are known to be of importance to marine life. Attempts to correlate the occurrence of a certain organism with an important marine process frequently led to generalizations which were later found to be totally unjustified.

If any phase of biology needs continuous study, it is that of bacteriology, because one deals here with living organisms, rather than with dead specimens, and one must study physiological processes rather than morphological structure. One can not bottle up some sea-water for 6 weeks or even days, then bring it to the laboratory and expect that the numbers and types of bacteria will have remained the same as under the natural conditions of the sea. One can not preserve bacteria, as one does animals or plants, then identify them, as an opportunity arises, since in most cases the bacteria are identified not by their appearance but by their activities.

The fact that a certain bacterium is active in a specific transformation in the laboratory is no proof as yet that it will be responsible for the same reaction in the sea. Samples of sea-water or of bot-

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SAMPLING TUBE

FOR COLLECTING, AT DIFFERENT DEPTHS, SAMPLES OF WATER UNDER STERILE CONDITIONS.

tom material removed from their environment and brought into the laboratory may give, within a very short period of time, frequently within a few hours, a bacterial population which is quite different quantitatively from the corresponding population in the sea itself; a number of factors may be responsible for this change.

In the case of the bacterial population of the sea, as in the case of soil bacteria or bacteria in inland waters and in sewage, one deals with mixed populations and not with pure cultures. It is not merely sufficient to isolate an organism, cultivate it and determine what it does in pure culture; it is far more important to determine what it does in its natural substrate and how these activities dovetail with the activities of other bacteria, as well as with the whole complex mass of higher plants and animals.

In order to illustrate the possible confusion that may arise from the investigation of a single bacterial process in the sea, it is sufficient to call attention to the study of the rôle of bacteria capable of reducing nitrate in certain important marine processes. The hypothesis of Drew concerning the precipitation of $CaCO_3$ in the tropics and that of Brandt concerning the insufficient plankton development in tropical seas, as compared with that of temperate and arctic regions, were based largely upon this one specific bacterial process. Not only was considerable importance attached to it, disregarding thereby numerous other chemical and bacteriological processes involved, but an attempt was even made to set up highly generalized theories concerning the whole system of metabolism in the sea on the basis of this process.

Drew has shown in 1913 that, in a sea-water medium containing nitrates and calcium salts of organic acids, precipitation of calcium carbonate takes place. This reaction was found to be a result of the reduction of nitrate by certain bacteria, which use the organic acid radicle as a source of energy. On the basis of this experiment, the theory

THE LABORATORY BOAT *ATLANTIS*

LEAVING FOR THE GULF OF MAINE FOR A SERIES OF CHEMICAL, BACTERIOLOGICAL AND PLANKTONOGRAPHIC INVESTIGATIONS.

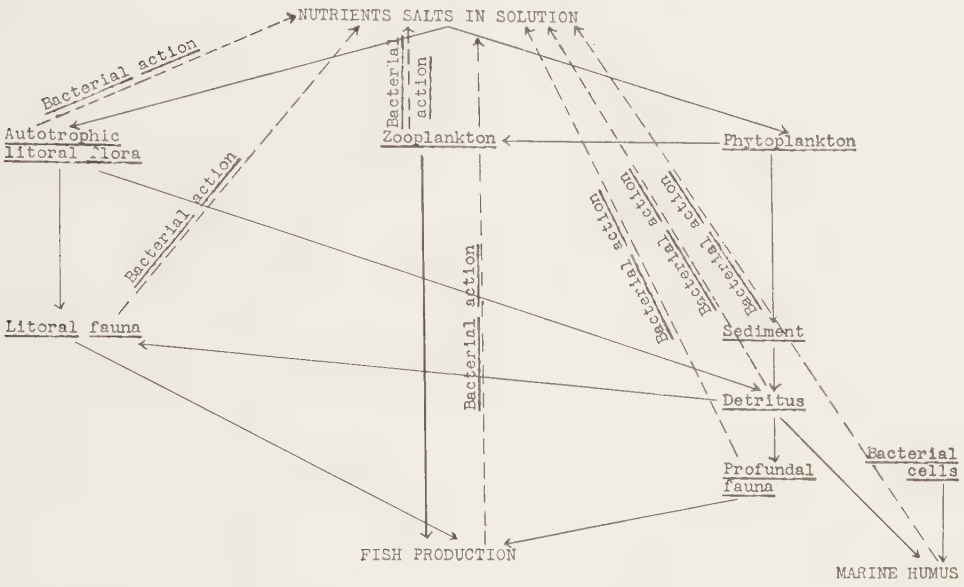


CHART I. PLANT, ANIMAL AND BACTERIAL RELATIONSHIPS IN THE SEA

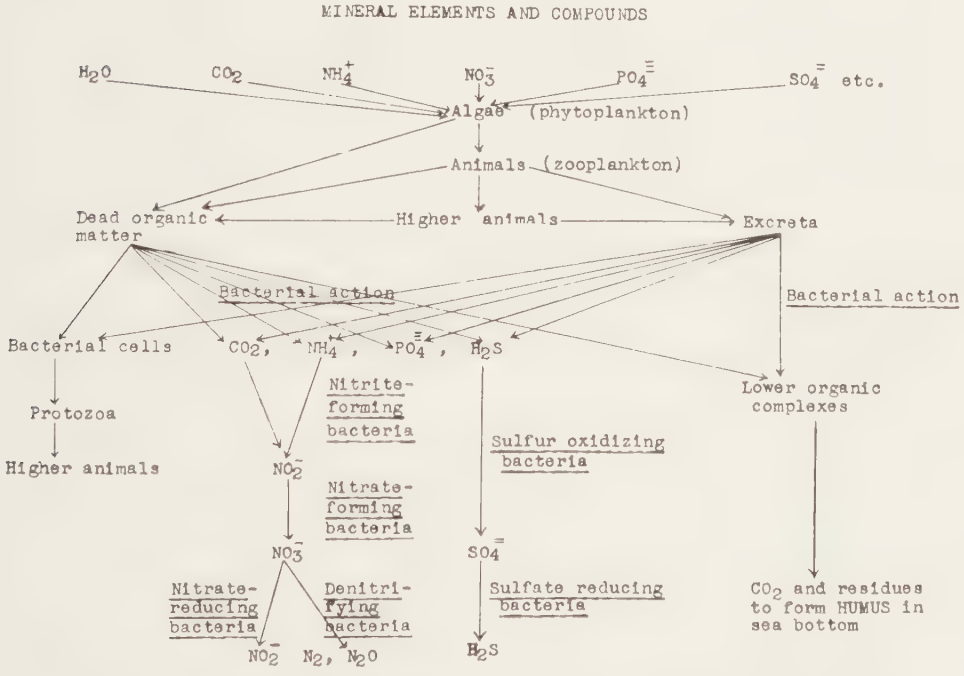
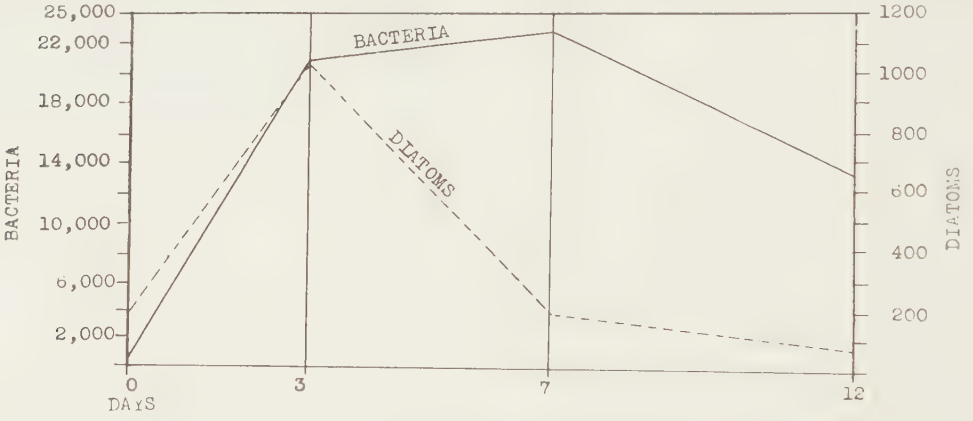


CHART II. RÔLE OF BACTERIA IN THE CYCLE OF LIFE IN THE SEA

was proposed that the precipitation of $CaCO_3$ in the chalk muds in the Bahama region, off the coast of Florida, and probably in other places is due to action of bacteria. Even granting that Drew was right in his conclusions, namely, that his experiments proved that precipitation of $CaCO_3$ in the sea may be due to the action of bacteria, he made two purely hypothetical assumptions not based upon any experimental evidence, which would tend to nullify these conclusions, as applied to large regions. These assumptions are that nitrates are produced in the sea in sufficiently large amounts and that sufficient quantities of organic acids are present in sea-water to bring about the dissolution of the calcium and to serve as an energy source to the nitrate-reducing bacteria. The mere fact that an organism can reduce nitrate in an artificial culture medium is no proof that this process forms an important phase of the activities of the organism under natural conditions. A great many pathogenic bacteria are able to reduce nitrate, although this substance does not occur in appreciable quantities in the blood or in other tissues where these bacteria are active.

In criticizing this hypothesis, C. B. Lipman suggested that the sea does not contain a sufficient number of bacteria to bring about such an extensive process. However, the activities of the bacteria are not limited by their numbers but by the amount of available energy, the abundance of nutrients and favorable environmental conditions. Lipman concluded that calcium precipitation in large quantities in the sea can be more readily explained by purely physicochemical processes, although he did not deny the possibility that microorganisms may bring about, directly or indirectly, the precipitation of some calcium carbonate. Kellerman and Smith, who upheld Drew's hypothesis, believed that the denitrifying bacteria are specific "calcium bacteria," indicating thereby that the process of calcium precipitation plays the predominant function in the life activities of these organisms. Molish went even a step further and described various new species of bacteria on the basis of their ability to cause the precipitation of calcium. These conceptions are hardly justified, since the reaction of calcium precipitation, even under Drew's experimental conditions, is only secondary in nature and does not form an important phase of the physiology of the bacteria. Further, in addition to various denitrifying bacteria, other organisms, such as urea and sulfate-reducing bacteria, are also able to bring



CHANGES IN NUMBERS OF BACTERIA AND DIATOMS IN SEA-WATER CULTURES; NUMBERS IN 1 CC OF CULTURE.

about the precipitation of calcium. Unfortunately, neither Drew nor those following him seemed to have been familiar with the fundamental work of Nadson on "Bacteria as Geological Agents" published nearly 30 years ago, which tended to illuminate this process.

The second hypothesis based upon the activities of nitrate-reducing bacteria was even more far-reaching in its application. Brandt was among the first to emphasize the important part played by bacteria in the cycle of life in the sea. He suggested the following explanation for the relative abundance of plankton in the arctic and temperate regions and its comparative poverty in the tropics. In warm regions, the denitrifying bacteria are very active and destroy the nitrates, thus bringing about a nitrogen minimum; in cold regions, where the denitrifying bacteria are not so active, the abundance of nitrate permits considerable plankton development. This hypothesis has had a considerable influence upon the subsequent development of research on the bacteria of the ocean. Assuming that the observations concerning the relative abundance of the plankton in the sea, under different climatic conditions, are correct, the explanation suggested is very much open to criticism, since one would have to establish first that nitrates are formed in the tropical regions as abundantly as in the temperate regions, that bacteria are more active in the tropics and that more energy, in the form of plant excreta or residues, is available in the tropical seas to enable those bacteria to reduce the nitrate. Possibly the more rapid disappearance of the organic residues in the tropical waters, due to the greater activity of the bacteria decomposing the organic matter, may have more to do with this phenomenon than the activities of the denitrifying bacteria. Recent studies by Butkewitsch established the fact that nitrate-reducing bacteria are

CONNECTING SAMPLING TUBE TO LINE

TUBE FOR SAMPLING OF MARINE MUD UNDER STERILE CONDITIONS

also found in cold regions as in the Barents Sea, while investigations carried out at the Woods Hole Oceanographic Institution brought out the fact that the activities of most of the nitrate-reducing bacteria in the sea are limited to the reduction of nitrate to nitrite and not to atmospheric nitrogen.

These two illustrations are sufficient to emphasize how certain limited observations on the activities of one group of bacteria isolated from the sea, namely,

PETERSEN GRAB
FOR SAMPLING OF MARINE BOTTOMS.

the denitrifying organisms, can be so magnified as to make them appear as among the most important members of the bacterial population of the sea. One should add here that Brandt's hypothesis was not accepted by Gran and other prominent oceanographers, who demonstrated that the available evidence is either insufficient or even contrary to it.

The Cycle of Life in the Sea and Bacterial Activities

A graphic representation of the rôle of bacteria in the cycle of life in the sea is given in Charts I and II. Chart I shows the general relation of the bacterial activities to the interrelationships of plants and animals in the sea and to the various degradation products, while a more detailed analysis of the specific marine processes in which bacteria are chiefly concerned is shown in Chart II.

According to Thienemann, the plankton consists of three groups of organisms: (1) "producers," comprising largely the chlorophyll-bearing diatoms and algae which build up organic substances from the inorganic nutrients dissolved in the water; (2) "consumers," or the animal representatives of the plankton which feed upon the living or dead members of the phytoplankton or their metabolic products; (3) "reducers," comprising the bacteria, which decompose and mineralize the high-molecular compounds formed by the producers and consumers. The last two groups are in definite nutritional relations to one another. One can easily enlarge upon this idea and include among the consumers also the higher animals, which feed both upon producers and consumers of a lower order; among the reducers one can include not only the bacteria of the plankton but also the bacteria of the sea bottom which take an active part in the destruction of plant and animal residues in the sea, as well as the animal population of the sea. Certain groups of bacteria will also be found among the producers, such as the nitrogen-fixing bacteria; bacteria may also be considered as consumers, since they synthesize bacterial cell substance. One can add a fourth group, namely, "transformers," which comprise those bacteria, like the nitrifying, sulfur-oxidizing, nitrate-reducing and others, which play a most important rôle in the transformation of various elements and compounds, aside from the above three general processes.

The plant population of the sea synthesizes, under proper light conditions, organic substances out of the simple nutrients, namely, the water, CO_2, available nitrogen compounds, soluble phosphates and other inorganic salts. The animals of the sea which are unable to utilize the photosynthetic energy of the sun must obtain their energy and materials necessary for the construction of their body substance from the plants, either directly or indirectly. Some lower animals, such as certain foraminifera, radiolaria, coelenterata and others, actually carry living algae which synthesize organic matter and thus serve for the nutrition of their host. After the death of the numerous plants and animals which inhabit the sea, their bodies as well as their excretion products are decomposed by bacteria living in the water, on the plankton or in the bottom sediments, as shown in the photographs.

DECOMPOSITION OF *ULVA LACTUCA* BY FUNGI, FOLLOWED BY ATTACK OF THE FUNGUS MYCELIUM BY BACTERIA.

Among the most important activities of the bacteria in the sea are the decomposition of plant and animal residues and the transformations of nitrogen. By decomposing the residues of marine plants and animals as well as their waste products, the bacteria return, in a mineralized form, to circulation in the sea, and in the atmosphere those elements from which the diatoms and algae inhabiting the sea synthesize their cell substance, namely, the carbon as carbon dioxide, the nitrogen as ammonia, the phosphorus as phosphate, etc. Without these actions of the bacteria, the sea bottom would soon become covered with a mass of dead plant and animal débris, and the limited supplies of available carbon (CO_2), of available combined nitrogen and of phosphate would soon become transformed into organic forms which are unavailable for growth of marine plants; this would result in cessation of plant life in the sea. In the process of decomposition of the residues, the bacteria themselves synthesize considerable cell substance, which may actually serve as food for various invertebrate animals and thus form a constituent part of the plankton of the sea.

Bacteria may also live symbiotically with various marine plants and animals. Among these symbiotic relationships, that of the green algae and the nitrogen-fixing bacteria has been suggested. In recent investigations carried out at the Woods Hole Oceanographic Institution, a close parallelism was found in the water and in the plankton between the abundance of diatoms and other plant and animal constituents and the numbers of bacteria. When the diatoms die, as shown by the rapid diminution in numbers, the bacteria still continue to develop at the expense of the diatom cells. This suggests the probability that the bacteria live in the free water only to a limited extent, but that they are largely associated with the plant and animal forms of the plankton, attacking

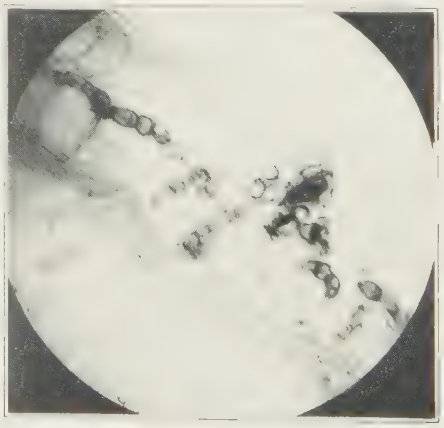
their excretion products, as well as the dead bodies.

Methods of Study of Marine Bacteria

Studies of marine bacteria have four distinct ends in view: (1) determination of the relative abundance of bacteria in the sea-water and in the sea-bottom material; (2) detection of the specific nature of the bacterial population, namely, the types of organisms that make up this population under the various conditions; (3) determination of the physiological activities of the bacteria isolated from the sea; (4) interpretation of the rôle of these bacteria in the cycle of life in the sea itself.

In the study of the occurrence, abundance and activities of bacteria in such a medium as the sea, in which they occur not in pure culture but as mixed populations, the methods employed are of considerable importance. These methods are both direct and indirect. In view of the small size of the bacterial cell, the relatively extreme dispersion of the bacteria and their comparatively low numbers in the ocean, any direct examination of the sea-water can tell very little concerning the occurrence and abundance of the bacteria; these cells should be concentrated, by removing much of the water, either by centrifugation, by filtration or by evaporation, before a microscopic examination is attempted. Most of the methods commonly employed in the past for the study of the abundance of marine bacteria were cultural in nature; here belong the gelatin and agar plate methods, the dilution method and the enrichment culture methods. Since no single medium will allow the development of all the bacteria present, even in a small quantity of sea-water or sea-bottom material, various media have to be employed. The early investigators limited themselves to counting bacteria by means of the plate method. At first the gelatin media were employed, soon followed by agar media, such as sea-water gelatin or sea-water fish extract gelatin and sea-water agar, with various modifications. It was recognized, however, at an early date that the sea contains many bacteria which are unable to develop on the culture plate. This led to the adoption of various dilution methods and specific enrichment culture methods. More recently there has been a tendency to use direct microscopic methods for the enumeration of bacteria both in the sea-water and in the sea bottom.

The plate method presents certain advantages but also various distinct limitations. The fact that one can determine the numbers of bacteria which are actually present in the sea in a living state, after they are allowed to develop on the plate, and the fact that one can isolate these bacteria from the plate for further study of their morphological and physiological characteristics are distinct advantages of the method. However, not all the bacteria present in the sea are able to develop on the common plate, some of the most important organisms, such as the nitrifying, sulfur-oxidizing, nitrogen-fixing, not developing at all; but even those bacteria that are able to grow on the plate will not all



CROSS-SECTION OF *ULVA LACTUCA*
SHOWING FUNGUS INVASION OF TISSUE; STAINED WITH HAEMOTOXYLIN AND CONGO RED.

develop and form colonies. The plate method may thus give an unbalanced picture of the bacterial population of the sea. To meet these objections, Cholodny and others suggested the use of a direct microscopic method. This method has certain advantages, since it gives a more correct picture of the abundance of bacteria in the sea; however, it does not enable one to distinguish the living from the dead bacteria or to separate the various types of organisms for further study of their physiological characteristics.

To determine the specific nature of the bacteria, the enrichment or elective culture method is usually employed; the sea-water or bottom material is added to a medium of specific chemical composition and the nature of the transformation brought about in the medium is determined. This method is very convenient for the purpose of demonstrating the occurrence of specific bacteria in the sea. By combining the dilution method with that of elective culture, the relative abundance of the specific bacteria can be estimated. This method was used for the purpose of demonstrating and isolating some of the most important bacteria now known to be found in the sea. The solution medium can be replaced by the silica-gel medium, which has found application in various investigations recently carried out at the Woods Hole Institution.[2]

No single method can be used for the study of the bacterial population of the sea as a whole. Various methods have to be combined in order to obtain an insight into the nature and abundance of this population. For the study of the abundance of proteolytic bacteria, the gelatin plate may be used, for agar-liquefying bacteria—the agar plate, for cellulose-decomposing bacteria—the silica gel cellulose plate, for demonstrating the presence of various other specific bacteria—various special methods have to be adapted.

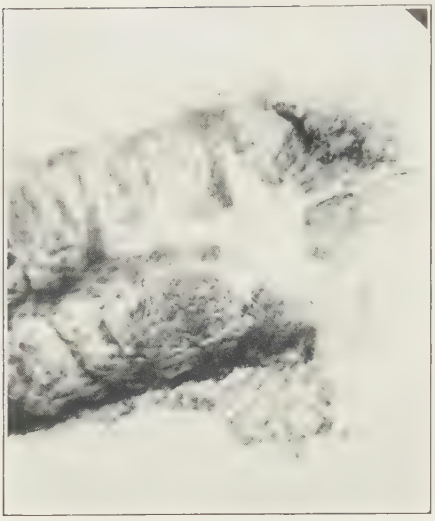
[2] S. A. Waksman, C. Carey, and H. W. Reuszer, *Biol. Bull.*, August, 1933.

DECOMPOSITION OF *ULVA LACTUCA* BY BACTERIA IN SEA-WATER MEDIUM; THE EXTREME RIGHT SHOWS THE THIN GRANULAR FILM OF BACTERIA ATTACKING THE EDGE OF THE ALGA.

For study of the biochemical activities of marine bacteria, methods are employed which are based both on the nature of the organism and upon the specificity of the process under consideration. These methods are in most instances adaptations of methods employed in general bacteriology. The most important phase of the subject, however, namely the study of the rôle of the bacteria in marine transformations under natural conditions, has scarcely been considered at all.

Nature of the Bacterial Population of the Sea

Fungi are either entirely absent in the sea or are found at infrequent intervals. Whenever reference is made to their occurrence in the sea, a closer examination of the data will reveal the fact that this was due either to contamination in



MAGNIFIED SECTION

OF DECOMPOSITION OF *Ulva lactuca* BY BACTERIA, ×900; STAINED WITH HAEMOTOXYLIN AND CONGO RED.

the process of the preparation of the plates or to their chance infiltration from land and from dust (wind), especially at places close to shore. The fact that the genera Aspergillus and Penicillium occupy a prominent place among the fungi which were thus commonly reported adds further weight to the above conclusion, since these genera comprise the common dust and wind-borne fungi. One need not exclude, however, the possibility that fungi play a part in the decomposition of algal material and Zostera, when exposed to the atmosphere, as shown in the figures. Actinomyces are also either entirely absent or infrequently observed. Yeasts have frequently been found, especially certain Torulae, but their rôle in marine processes has been studied but little.

The bacterial population of the sea is quite characteristic. It is distinct in nature from the population usually found on land, as shown by the more limited number of bacterial types found in the sea. Spore-forming bacteria, which comprise an important part of the bacterial population in soil, are practically absent in sea-water, although they may be present in considerable abundance in the sea bottom. Cocci are also of limited occurrence in the sea. Motile rods and various types of vibrios, or comma-shaped organisms, usually make up the major part of the bacterial population thus far studied. The poverty of bacterial species in the sea depends largely upon the specific nature of sea-water as a medium for the growth of these organisms.

Certes observed in 1884 that only aerobic bacteria are present in the ocean; no anaerobic forms were noted. Bacteria were found to occur in the water to very considerable depths, namely, 5,100 meters. Fischer determined that, on an average, 1 cc of sea-water contains 1,083 bacteria, with a maximum of 29,400 and a minimum of 0. More bacteria were found in the winter than in the summer months. The marine bacteria were shown to be morphologically and physiologically distinct from the land forms, most of them being curved or spirilloid in shape.

In 1892, Russell demonstrated that sea mud contains greater numbers of bacteria than the above lying water: at a depth of 50 meters, there were only 121 bacteria in 1 cc of water, while 1 cc of mud contained 245,000 bacteria. Russell believed that the bacteria settle upon the mud in the form of spores or vegetative cells from the upper layers of water. The nature of the mud and its depth were found to influence the number of bacteria considerably. Most of these bacteria were proteolytic and were able to reduce nitrate to nitrite. According to Bertel, away from the coast in the open sea, the bacteria increase in numbers with depth. A marked difference in the numbers of bacteria in sea-water and in sea mud was also reported by Smith, Bavendamm and others. As many as 16,000,000 bacterial cells were

found in 1 gram of mud from the mangrove swamps of the Bahamas. Many of these bacteria were strongly agar-liquefying, pointing to their capacity of decomposing the organic complexes which are synthesized so abundantly by the algae in the sea.

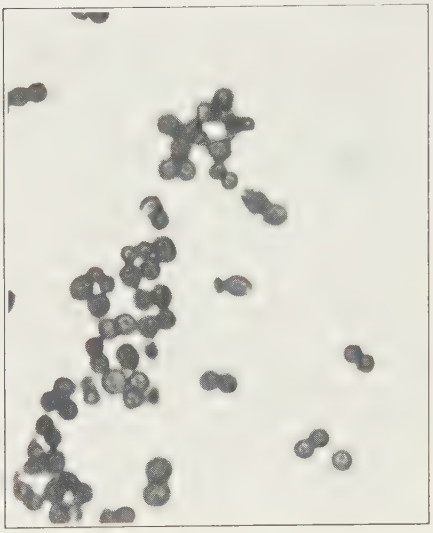
In a recent study carried out at the Woods Hole Institution,[3] it was found that in ocean waters close to land and at sufficient depth to preclude agitation of the bottom by currents and wave motions, the numbers of bacteria are very small, usually not more than a few cells per 1 cc of water. The depth of the water and the nature of the sea bottom are of importance in this connection. Along shores receiving considerable land drainage, the numbers are much greater. The bottom deposits, especially the mud bottoms, are much richer in bacteria than the waters. A marked parallelism was found between the plankton organisms and the abundance of bacteria in the water, as well as a definite relation between the organic matter or humus content of the mud and the abundance of bacteria.

Among the specific bacteria commonly found in the sea, the nitrifying and denitrifying organisms have attracted particular attention. The formation of nitrates in the sea and their disappearance have aroused considerable interest. It was at first believed that the nitrates are brought into the sea from land or by atmospheric agencies. However, more recent evidence seems to point to the fact that nitrate formation is largely a result of bacterial activities in the sea itself. Thomsen and later Issatchenko, Lipman and others established the fact that, although nitrifying bacteria are not found in the surface waters of the sea, they can be readily demonstrated in the sea bottom. The investigations carried out at the Woods Hole Institution seem to point in the same direction.

As to the bacteria capable of reducing nitrate in the sea, one must avoid confusing nitrate-reducing bacteria with denitrifying bacteria: the first may reduce the nitrate only to nitrite and possibly to ammonia, while the latter break down the nitrate to atmospheric nitrogen or to gaseous forms of nitrogen. Although nitrate-reducing bacteria are present abundantly in the sea, denitrifying bacteria are found there but seldom, except possibly near shore or in the sea bottom. The results of Issatchenko, Butkewitch and those obtained recently at Woods Hole point in that direction. The mere fact that nitrite can be formed from nitrate by bacteria in the sea is no proof as yet that it leads to losses of nitrogen, since the nitrite can be assimilated by the phytoplankton organisms in their nutrition.

The presence in the sea of bacteria capable of bringing about the fixation of nitrogen has also attracted considerable attention. Benecke and Keutner were the first to isolate from the sea the aerobic *Azotobacter* and the anaerobic *Clostridium pastorianum,* the first organism occurring largely in the plankton and the second in the lower layers of water. Keutner, Keding and Issatchenko later demonstrated that Azotobacter cells are found on various algae growing in the sea; the same was frequently true of Clostridium and of accompanying bacterial forms. Reinke believed that a certain form of symbiosis exists between the algae and the Azotobacter, similar to that of the nodule bacteria and the leguminous plants. A typical culture of the aerobic, nitrogen-fixing bacterium, *Azotobacter chroococcum,* isolated from the sea-water, in the neighborhood of Woods Hole, is shown in the accompanying figure. Unfortunately, the importance of these organisms in ocean life has not yet been definitely established. Their occurrence in sea-water and in the sea bottom under different conditions, at

[3] H. W. Reuszer, *Biol. Bull.,* 1933. (In press.)



AZOTOBACTER CHROOCOCCUM

THE AEROBIC NITROGEN-FIXING ORGANISM, ISOLATED FROM THE SEA, ×1650.

different depths and at different temperatures, as well as the conditions influencing their activities, still remain to be determined.

Among the bacteria capable of bringing about the decomposition of marine residues, the agar-liquefying organisms, which are particularly abundant in the phytoplankton, have attracted considerable attention. These bacteria are capable of producing an enzyme which liberates soluble sugars from the complex carbohydrates which form the important constituents of the marine plants. The occurrence in the sea of cellulose-decomposing, of fat-splitting, of proteolytic and of various other bacteria has also been established.

Decomposition of Plant and Animal Residues and Transformation of Nitrogen

In order to understand the mechanism of the decomposition of the organic residues, it is important to know their chemical nature. Among the members of the phytoplankton in the sea, the diatoms are most abundant in the colder regions, while the Peridineae and the Schizophyceae are present largely in the warmer regions. The chemical composition of several members of the phyto- and zooplankton has been reported by Brandt as follows:

Chemical constituents, per cent. of dry material	Nature of organism		
	Chaetoceras	Ceratium	Copepods
Protein	10.0	13.0	59.0
Chitin	.....	.....	4.8
Fat	2.8	1.3	7.3
Soluble carbohydrate	22.0	39.0	22.5
Cellulose	0	41.5	0
SiO_2	54.5		
Salt and other ash materials	10.7	5.2	9.2

In general, the organic carbon of the plankton is found to range from 14 to 48 per cent., the nitrogen from 1.5 to 10.6 per cent., and the ash from 13 to 65 per cent. The diatoms are richest in ash and lowest in nitrogen, while the zooplankton is low in ash and high in protein. Only the Peridineae contain cellulose, while the hemicelluloses form the most abundant group of carbohydrates among the diatoms and the various lower and higher algae. Fats are present abundantly, while the nature of the proteins still remains to be studied. It is of interest to note that 1 gram of dry matter in the plankton corresponds to 500,000 copepods, to 65,000,000 peridinia and to 675,000,000 diatoms.

It is of interest to compare the above analyses with that of a typical marine alga, namely, *Fucus vesiculosus* (see table on p. 47).

When the dead plants and the animals, their excreta, the various residues and degradation products undergo decomposition in the sea by the action of the bacteria, a number of organic and mineralized complexes are formed. A part of the organic substances may become soluble in the sea-water, while

Chemical constituents	Per cent.
Protein	5.4
Fat	2.3
Alcohol-soluble material	6.7
Cold water-soluble organic matter	13.6
Hot water-soluble organic matter	7.8
Pentosan*	28.7
Uronic acid anhydride*	27.5
Cellulose	3.9
Ash	13.9

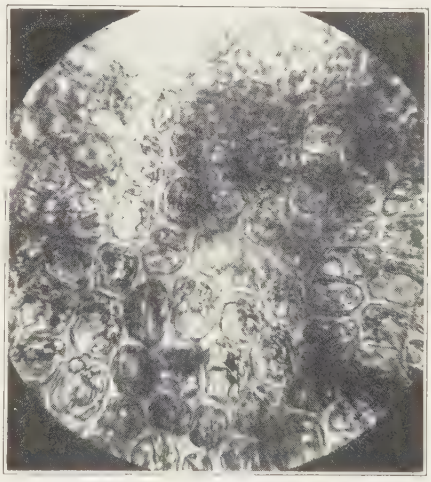
* These two fractions represent largely the same chemical complex determined by two different procedures.

some of the residual substances as well as the synthesized bacterial cells may drop to the bottom and contribute to the formation of the marine humus.

The most important compounds formed in the process of decomposition of the marine plant and animal residues are ammonia, carbon dioxide and phosphates; the ammonia is oxidized by certain specific bacteria to nitrite, and then to nitrate. These compounds are thus made available for the nutrition of the marine plant population, namely, the phytoplankton and the algae. Raben and a number of other investigators demonstrated the fact that the deep water in the open ocean contains more nitrate and phosphate than the upper layers in which phytoplankton organisms are active; the North Sea waters contain more nitrate and phosphate in the summer than in the winter months. According to Brandt, the nitrate of the sea is continually renewed by rain and by rivers; he suggested that the denitrifying bacteria reduce the concentration of nitrates in warm regions, which thus accounts for the reduced phytoplankton production. Harvey concluded, however, that the introduction of nutrients from land into sea plays only a minor part, when compared with the quantity of nutrients formed yearly from the dead organic residues in the ocean itself. After death, the algae and the animal members of the plankton sink to the bottom and are decomposed there by bacteria, with the result that the phosphate and nitrogen are liberated in an available form. These mineralized nutrients are then brought to the upper layers by vertical circulation. The bacteria are thus recognized as playing a most essential function in the cycle of elements in the sea.

Organic Matter in Sea-Water and in the Marine Bottom

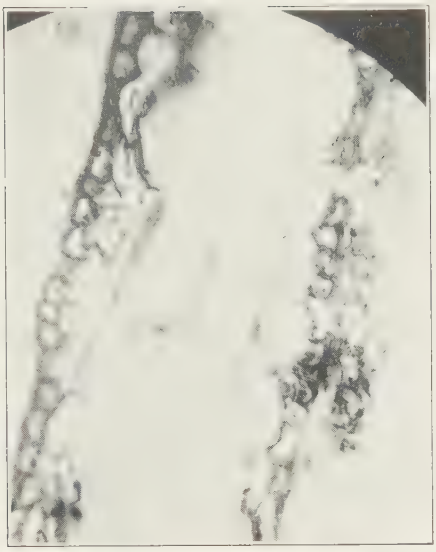
The question of the abundance, chemical nature and function of the organic matter found in the sea in an unorganized form, either in solution in the water or in a solid state on and in the



ZOSTERA

HEALTHY LEAF, SURFACE VIEW, × 600.

sea bottom, presents a number of highly important problems in which the activities of the bacteria play a prominent part. The function of the dissolved organic matter in the nutrition of the marine animals has been emphasized by Pütter. This theory was submitted to considerable criticism by Krogh and others. Pütter's figures for the dissolved organic matter in the sea were shown to be too high, and some of this organic matter was shown to be due to

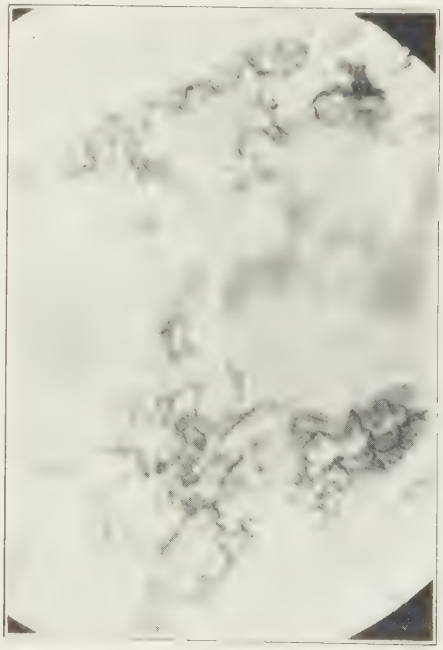


ZOSTERA LEAF
AFTER 30 DAYS OF DECOMPOSITION, ×400.

the suspended bacteria, protozoa and unicellular plants present in the water. Although a part of the organic matter in solution may be liberated by the algae, there is no doubt that another part is produced by the bacteria in the process of decomposition of the plant and animal residues in the ocean.

Another source of organic matter in the sea is the marine bottom. This organic matter can be designated as "marine humus," because of its similarity in chemical nature, and probably in origin, to the humus found in soils. The concentration of this humus in the marine bottom varies considerably, namely, from 0.5 per cent. in the case of sand bottoms to nearly 10 per cent. in the case of certain mud bottoms. In some localities, such as protected harbors or fjords, the mud bottom may contain as much as 20 per cent. humus. This humus is best calculated from the organic carbon content, by the use of a certain factor. Boysen-Jensen suggested the use of a factor of 2, assuming that the carbon content of the humus is 50 per cent. However, a study of the chemical composition of the humus brought out the fact that this factor should be lower than 2, namely, 1.923, due to the presence in the humus of lignin-like complexes with a high carbon content.

One of the most important characteristics of this marine humus is its carbon-nitrogen ratio. Boysen-Jensen found this ratio to vary from 8:1 to 12:1, with an average of about 10:1. These results were fully confirmed by other investigators. When one recalls the fact that a similar ratio of carbon to nitrogen holds also for soils, the similarity of the marine humus to soil humus becomes even more apparent. Organic complexes similar to those found in soil humus can also be isolated from the marine humus. There are several reasons for the similarity: (1) a part of the marine humus is of terrestrial origin, being brought into the sea by streams and rivers; (2) the marine humus, just as the soil



ZOSTERA LEAF
AFTER ADVANCED DECOMPOSITION, ×400.

humus, is formed by similar microorganisms in a very similar manner. Certain differences observed in the chemical nature of the humus can easily be accounted for by the fact that marine residues, from which all or part of the humus is formed, are different in chemical composition from the land residues from which all the soil humus is formed.

The organic matter in solution in the sea-water as well as the humus in the mud are only slowly acted upon by bacteria; otherwise neither form of organic matter could be present in great abundance in the sea. However, even their slow decomposition is sufficient to provide a certain amount of available energy and nutrients for various bacteria living in the water and especially in the sea bottom.

Truly, the life of the plant and animal world in the ocean is variously dependent upon the manifold activities of the oceanic microscopic population composed principally of bacteria.

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Über den Gesamtphosphorgehalt des Seewassers im westlichen Nordatlantischen Ozean.

Vorläufiger Bericht von **H. R. Seiwell** und **G. E. Seiwell** [1]).
Beitrag Nr. 25 Woods Hole Oceanographic Institution.

(Hierzu Tafel 2.)

Vorwort.

Die Beobachtungen, welche diesem vorläufigen Bericht zugrunde liegen, stammen von einer Fahrt der „Atlantis“ im Nordwest-Atlantischen Ozean von Februar bis März 1932. Die Bestimmungen des Phosphatphosphors wurden an Bord, die des Gesamtphosphors nach der Rückkehr im Ozeanographischen Institut in Woods Hole ausgeführt. Die im folgenden wiedergegebenen Ergebnisse dürften die ersten ihrer Art sein, die aus dem offenen Ozean für die Oberfläche und das Tiefenwasser veröffentlicht werden.

Die ersten Untersuchungen über den gesamten und den organisch gebundenen Phosphor wurden von der Limnologen-Schule von Wisconsin durchgeführt (Birge und Juday (4, 5)*). Diese Forscher untersuchten mit Hilfe der von ihnen entwickelten Methoden den gelösten und den gesamten Phosphor in 479 Seen des nordöstlichen Wisconsin (U. S. A.).

Bestimmungen des Gesamtphosphorgehalts des Seewassers in der Nordsee und an der Küste von Island wurden von Kalle (6) 1933 veröffentlicht. Diese Beobachtungen wurden im September 1932 angestellt und beziehen sich ausschließlich auf Oberflächenwasser.

Einleitung.

Die bisherigen Untersuchungen über die Fruchtbarkeit des Meeres gründen sich fast ausschließlich auf die Untersuchung der anorganischen, im Seewasser

[1]) Dieser Bericht wurde im Institut für Meereskunde zu Berlin geschrieben. Wir danken Herrn Prof. Defant, der uns freundlicherweise Arbeitsmöglichkeit in seinem Institut gewährte, sowie Herrn Dr. Wattenberg, mit dem wir Gelegenheit zu vielen Diskussionen hatten.

*) Diese und die folgenden Ziffern beziehen sich auf das Literaturverzeichnis am Schluß dieser Abhandlung.

gelösten Nährsalze. Diese Substanzen, besonders die Phosphor- und Stickstoffverbindungen, werden in den Oberflächenschichten natürlicher Gewässer von den Pflanzen bei der Assimilation verbraucht. Die anorganischen Salze werden in anorganische Verbindungen verwandelt und werden erst nach dem Tode und nach der Zersetzung der Organismen wieder abgebaut und im Wasser aufgelöst. Falls die Regeneration und Wiederauflösung der Nährstoffe in Tiefen vor sich geht, die unterhalb der Assimilationszone liegen, werden diese Substanzen ganz oder teilweise in die Oberflächenschicht zurücktransportiert, wo sie erneut verbraucht werden können. Die zu einer bestimmten Zeit in der Assimilationsschicht vorhandene Menge anorganischen Phosphors (Phosphat) ist nur eine sekundäre Größe. Diese Menge kann aufgefaßt werden als das Ergebnis des Gleichgewichts zwischen der pro Zeiteinheit zugeführten und verbrauchten Menge.

Man kann daher nicht immer die Fruchtbarkeit von Meeresgebieten auf Grund der Phosphatphosphor-Konzentration (oder des Gehalts an irgendeinem anderen Nährstoff) beurteilen, welche in der Assimilationsschicht zu einer bestimmten Zeit vorhanden ist. Eine Gegend mag eine reichliche, dauernde Zufuhr von Nährstoffen in der Oberflächenschicht haben, und trotzdem kann das Gleichgewicht bei sehr niedrigen Nährstoffgehalten liegen, wenn sehr günstige Assimilationsbedingungen herrschen.

Die Beziehung zwischen Plankton und Phosphat in der Oberflächenschicht (0 bis 50 m) im Südatlantischen Ozean ist von Hentschel und Wattenberg (3) diskutiert worden. Diese Autoren fanden, daß im allgemeinen eine gute Übereinstimmung zwischen Phosphatzufuhr und Planktongehalt besteht in der Weise, daß bei hohen Phosphatkonzentrationen meistens die stärkste Planktonbevölkerung auftritt. Andererseits gab es aber auch Gebiete, in denen eine solche Übereinstimmung nicht festzustellen war.

Es ist wahrscheinlich, daß manche der auffallenden und widerspruchsvollen Probleme des Kreislaufes der Nährstoffe sowie ihre Beziehung zur Fruchtbarkeit der See geklärt werden könnte, wenn man nicht nur die anorganischen, sondern auch die organischen Verbindungen der als Nährstoffe wichtigen Elemente untersucht. Im folgenden wollen wir kurz die Beziehungen zwischen dem anorganisch gelösten, dem organisch gebundenen und dem Gesamtphosphorgehalt des Wassers im westlichen Nordatlantischen Ozean betrachten.

Zunächst muß betont werden, daß der sogenannte „Gesamtphosphorgehalt" einer Wasserprobe, die mit einem Wasserschöpfer oder einem anderen Schöpfinstrument genommen wurde, nicht unbedingt den wahren Gesamtphosphorgehalt *in situ* wiedergibt. Die Gesamtphosphorwerte sollen ja sowohl die gelösten Phosphate, wie die gelösten organischen Phosphorverbindungen, wie auch den in Pflanzen, Tieren, Detritus usw. enthaltenen Phosphor erfassen. Wenn man Wasserschöpfer oder eine Pütze zum Sammeln oder Proben benutzt, so erhält man eine ziemlich quantitative Probe allen Phosphors, der in obigen Formen vorhanden ist, jedoch mit Ausnahme desjenigen Anteils, der in dem größeren Plankton und Tieren verkörpert ist. Die größeren Planktontiere sind nämlich häufig sehr stark rheotaktisch und weichen vor der Öffnung des Schöpfers zurück. Wenn man daher wirkliche Gesamtphosphorwerte für eine Wassermasse *in situ* haben will, so muß man außer der Wasserschöpferprobe auch noch quantitative Fänge des Makroplanktons besitzen und auch deren Gesamtphosphorgehalt bestimmen und zu dem der Wasserschöpferprobe addieren. Solche Bestimmungen machen einige Schwierigkeiten und wurden im vorliegenden Falle nicht durchgeführt. (Betreffs der quantitativen Planktonfänge sei auf die kürzlich erschienene Arbeit von H. H. Gran verwiesen.)

Wir können in erster Annäherung die Fruchtbarkeit verschiedener Meeresgebiete zu einer bestimmten Zeit auf Grund des Gehalts der Wasserschöpferproben an organisch gebundenen Phosphor vergleichen, selbst wenn auf diese Weise nur Minimumwerte erhalten werden. Die Anwesenheit von Makroplankton im Wasser wird den organischen Phosphor einer Wasserschöpferprobe vergrößern durch Detritus sowie durch gelöste phosphorhaltige organische Substanzen, welche beim Absterben und der Zersetzung der Organismen oder als Exkrete

ausgeschieden werden. Wenn der Gehalt des Oberflächenwassers an organischem Phosphor in einem Gebiet höher ist als in einem anderen, so ist zur Zeit der Beobachtung höchstwahrscheinlich die erste Gegend fruchtbarer als die zweite.

Andererseits geht aus dem oben Gesagten hervor, daß wegen der verschiedenen Formen, in denen der organische Phosphor vorliegt, bei allen Vergleichen zwischen organisch gebundenem Phosphor und Plankton nicht nur das mit dem Wasserschöpfer gefangene Phytoplankton bzw. Nannoplankton berücksichtigt werden muß, sondern die gesamte, auch die größeren Organismen umfassende Menge der Lebewesen.

Diese Beziehungen sind also grundsätzlich verschieden von denen, welche, wie von verschiedenen Autoren gezeigt wurde, zwischen Phosphatphosphor und Phytoplanktonproduktion bestehen. Es ist jedoch wahrscheinlich, daß eine unmittelbare Beziehung zwischen organischem Phosphor und Phytoplanktongehalt in solchen Gegenden anzutreffen ist, in denen zur Zeit der Beobachtung sehr wenig Makroplankton vorhanden ist.

In diesem Zusammenhang mag erwähnt werden, daß Kalle (6) die Gesamtphosphorwerte des Oberflächenwassers an der Ostküste Islands mit der Menge Zwergplankton verglichen hat, welche Hentschel (2) gleichzeitig bestimmte. Kalle fand, daß im allgemeinen ein Ansteigen des Gesamtphosphors mit einem Abfallen der Planktonkonzentration zusammenfiel und umgekehrt, und er vermutet, daß diese Beziehung darauf beruht, daß ein großer Teil der Planktonorganismen bereits abgestorben und durch Absinken in die Tiefe der Oberflächenschicht einen beträchtlichen Teil des Gesamtphosphors entzogen hat. Kalles Erklärung scheint uns nicht ganz hinreichend zu sein. Der Tatsache, daß er nur das Kleinplankton, in erster Linie das Phytoplankton, für seinen Vergleich verwendet, liegt die Vermutung nahe, daß die Beziehung zwischen Zunahme des Planktons und Abnahme des Gesamtphosphors und umgekehrt keine wirkliche Beziehung ist, sondern ein zufälliges Zusammentreffen. Hentschels (2) Planktonuntersuchungen für dieselbe Gegend weisen verhältnismäßig wenige Metazoen auf, während nach Paulsen (7) im Sommer überall rund um Island das Meer sehr reich an Makroplankton ist. Dieser Umstand läßt uns vermuten, daß Hentschels Methoden nur das Zwergplankton, nicht aber das größere Plankton erfaßten. Wenn das der Fall ist, so würde Kalle den Gesamtphosphor nur mit einem Teil des Planktons vergleichen, da die sicher vorhandenen Schwärme von Makroplankton unberücksichtigt blieben.

Möglicherweise ist das Anwachsen des Gesamtphosphors bei Abnahme der Nannoplanktonbevölkerung (und umgekehrt) so zu erklären, daß in den isländischen Gewässern die Kleinplanktonmenge durch die Makroplanktonschwärme stark aufgezehrt war. Dadurch würden, auch wenn das Makroplankton selbst zum größten Teil bei der üblichen Methode des Wasserschöpfens entweicht, genügend Detritus und Exkrete produziert werden, um den Gesamtphosphorgehalt des Wassers zu erhöhen. Ähnlich würde eine Zunahme des Planktons die Abwesenheit von Makroplankton und damit einen geringeren Gesamtphosphorgehalt bedeuten. Diese Erklärung bleibt indessen nur eine Hypothese, solange wir keine unsere Annahme unmittelbar beweisenden Beobachtungen besitzen.

Methoden.

Die Methode von Robinson und Kemmerer (8) zur Bestimmung des Gesamtphosphors in Süßwasserseen wurde etwas modifiziert und zu der Bestimmung des Gesamtphosphors im Meerwasser angepaßt. Das Verfahren sei im folgenden kurz beschrieben.

Da die Methode an Bord nicht anwendbar ist, wurden die Wasserproben in oft benutzten grünen Glasflaschen bis zur Untersuchung im Laboratorium aufbewahrt. Vor der Bestimmung wird der organische Detritus durch starkes etwa 15 Minuten langes Schütteln in einer Schüttelmaschine gleichmäßig in der Wasserprobe verteilt. Zu 50 ccm der Wasserprobe wird in einem 250 ccm/Erlenmeyer aus Geräteglas 0.2 ccm konzentrierte Schwefelsäure zugefügt und das Ganze auf ein Volum von 10 bis 15 ccm eingedampft. Nach dem ersten Eindampfen wird

0.5 ccm konzentrierte Salpetersäure und 3 ccm konzentrierte Salzsäure zugegeben und das Eindampfen fortgesetzt, bis bei ungefähr 170° C Dämpfe von SO_3 auftreten. Nach dem Abkühlen wird der Überschuß der Säure mit starkem Ammoniak neutralisiert, auf 100 ccm verdünnt und der Phosphatgehalt kolorimetrisch bestimmt.

Das beschriebene Verfahren ist nicht allzu zeitraubend, wenn man eine größere Anzahl Proben, z. B. 50 bis 100, auf einmal verarbeitet.

Kalle (6) empfiehlt für die Bestimmung des Gesamtphosphors im Meerwasser eine Methode, die auf dem modifizierten Winklerschen Verfahren zur Bestimmung von Albuminoid-Stickstoff aufgebaut ist. Diese Methode beruht darauf, daß der organisch gebundene Phosphor durch Erhitzen der Wasserprobe Schwefelsäure und Kaliumpersulfat aus dem Wasserbad in anorganisch gebundenen Phosphor übergeführt und als solcher bestimmt wird.

Ergebnisse.

1. **Oberflächenbeobachtungen.** In Tabelle 1 sind die Werte für Phosphat und organischen Phosphor (in Milligramm P pro cbm) von der Oberfläche des offenen westlichen Nordatlantischen Ozeans wiedergegeben (vgl. untenstehende Abbildung).

Tabelle 1.

„Atlantis" Station	N-Br.	W-Lg.	Temperatur Oberfläche	Phosphat-P mg/m³		Organischer P mg/m³	
				Oberfläche	0—50 m	Oberfläche	0—50 m
1154	34° 20′	51° 45′	17.76	2	2	6	8
1157	35° 10′	44° 40′	17.80	1	1	14	13
1160	30° 26′	42° 53′	20.38	1	2	10	9
1163	25° 53′	41° 25′	22.47	1	1	12	13
1166	20° 50′	41° 38′	24.26	1	1	13	13
1169	16° 22′	41° 10′	25.07	2	2	18	20
1172	11° 43′	40° 54′	25.60	0	0	20	19
1174	8° 20′	40° 45′	25.73	4	7	19	28
1179	0° 45′	42° 38′	27.50	3	3	19	—
1210	22° 14′	67° 50′	23.95	2	2	9	10
1214	25° 33′	66° 45′	21.88	2	2	12	12
1217	29° 55′	65° 15′	20.14	4	4	11	11
1222	33° 15′	67° 35′	20.69	1	2	14	13
1227	35° 18′	72° 47′	22.11	0	1	15	15

Ozeanographische Stationen der „Atlantis" im nordwestlichen Atlantischen Ozean, auf denen Bestimmungen des Gesamtphosphors im Februar und März 1932 ausgeführt wurden.

Die Oberflächenschicht der südlichen Hälfte des Nordwestatlantischen Beckens ist im allgemeinen arm an anorganischen Phosphaten, im Mittel wird eine Konzentration von 2 mg P/m³ in den obersten 50 m selten überschritten. Das Maximum finden wir auf Station 1174 in der Gegend des Nordäquatorialstromes mit einem Mittelwert von 7 mg P/m³ für die 0 bis 50 m-Schicht. Die Phosphatwerte der Tabelle 1 für die Oberfläche und die Schicht bis 50 m sind das Ergebnis des Gleichgewichts, das zwischen der Zufuhr von Nährstoffen und dem Verbrauch durch das Plankton besteht.

Wegen der in dieser Gegend durchweg herrschenden hohen Temperatur wird das Phosphat offenbar sehr schnell durch die Assimilation aufgebraucht, und das Gleichgewicht stellt sich bei sehr niedrigen Werten ein. Eine Ausnahme bildet die Station 1174, wo das nährstoffreiche Tiefenwasser dicht unter der Oberfläche liegt.

Man kann annehmen, daß die Werte für den organisch gebundenen Phosphor ein brauchbares Maß für die relative Fruchtbarkeit der Gegend zur Zeit der Beobachtung gibt. Im Nordwestatlantischen Ozean haben wir in den obersten 50 Metern nördlich von 20° N (bis 36° N) Konzentrationen von 8—15 mg P/m^3, südlich von 17° N bis zum Äquator dagegen Werte von 19–28 mg. Der höchste Wert liegt wieder auf Station 1174 im Nordäquatorialstrom, wo auch große Mengen anorganischen Phosphors für die Photosynthese zur Verfügung standen. Die Zweiteilung des Gebietes bestätigt frühere Schlüsse anderer Forscher, daß nämlich die Äquatorialzone von größerer Produktionsfähigkeit ist als das antizyklonale Subtropengebiet.

Die Oberflächenwerte von organischem Phosphor im nordwestlichen Atlantischen Ozean sind kleiner als die, welche Kalle (6) für das Oberflächenwasser der Nordsee und das Küstenwasser für Island erhalten hat. Dieser Autor fand Gesamtphosphorwerte bis zu 98 mg P/cbm (entsprechend 93 mg organischen Phosphors). Es ist natürlich zu erwarten, daß infolge der stärkeren Produktivität der nördlichen Küstengewässer während der kurzen Vegetationsperiode größere Mengen organischen Phosphors auftreten als zu irgendeiner Jahreszeit in den Topen. In einer früheren Arbeit wurde die Fruchtbarkeit der Barents-See und des tropischen Nordatlantischen Ozeans während der Produktionszeit verglichen [Seiwell (10)]. Die erhaltenen Mittelwerte führten zu dem Schluß, daß während der Vegetationszeit die produktiven Schichten der Barents-See 5 bis 6 mal fruchtbarer waren als die des äquatorialen Nordatlantischen Ozeans. Die Annahmen, auf die diese Berechnung gegründet war, hatten jedoch mehr hypothetischen Charakter.

2. Die vertikale Verteilung des organisch gebundenen Phosphors.

Tafel 2 zeigt die vertikale Verteilung des Phosphats, des organischen und des gesamten Phosphors bis zu 1000 m Tiefe für die Stationen 1154, 1169 und 1172, welche im östlichen Teil des westlichen Nordatlantischen Beckens liegen, und für die Stationen 1210, 1217 und 1222, welche in der westlichen Hälfte des Beckens liegen. Die Beobachtungswerte selbst sind in Tabelle 2 wiedergegeben. Die Stationen 1154, 1210, 1217 und 1222 sind typische Stationen der Sargasso See, die Station 1172 ist typisch für die Nordäquatoriale Zone. In unserer kurzen Diskussion betrachten wir nur die Wasserschicht bis zu einer Tiefe von 1000 m. Auf Grund der Ergebnisse einer früheren Arbeit über den Sauerstoffgehalt des westlichen Nordatlantischen Ozeans nehmen wir an, daß die Zersetzung organischer Substanz in diesem Teil des Ozeans im allgemeinen nur in den oberen 1000 Metern vor sich geht [Seiwell (9)].

Die vertikale Verteilung des organischen Phosphors ist in dieser Wasserschicht bis zu einem gewissen Grade reziprok der vertikalen Verteilung des Phosphatphosphors. Die geringsten Konzentrationen an Phosphat werden immer in den Oberflächenschichten gefunden, während die höchsten Werte für das Tiefenwasser charakteristisch sind; umgekehrt beim organisch gebundenen Phosphor, bei dem die Maximalwerte in den Oberschichten, die Minimalwerte im Tiefenwasser auftreten. Zwischen beiden Phosphorarten besteht eine allgemeine Beziehung. In der Oberflächenschicht wird das Phosphat von den Pflanzen bei der Assimilation verbraucht und in organische Bindung übergeführt. Die Pflanzen sterben entweder oder werden von Tieren verzehrt, welche sich in den unteren Schichten zersetzen, wobei anorganisch gebundener Phosphor wieder im Wasser gelöst wird. Wir nehmen an, daß der organische Phosphor in den Oberschichten deshalb so hohe Konzentration erreicht, weil das Plankton und das organische Leben überhaupt in dieser Schicht angereichert ist, während der Phosphor im Tiefenwasser in der Form von organischen Detritus, welcher von der Zersetzung der Organismen stammt, allmählich in die Phosphatform übergeführt wird. Das Ergebnis wird eine Abnahme des organischen Phosphors mit der Tiefe bei entsprechender Zunahme des anorganischen Phosphats sein.

Tabelle 2.

Tiefe	Phosphorgehalt in mg P/m: Phosphat	Phosphorgehalt in mg P/m: organisch	Gesamt P	Verhältnis $P_{Phosphat} : P_{organisch}$
Station 1154. 34° 20′ N-Br., 51° 45′ W-Lg.				
0	2	6	8	1 : 3
35	2	8	10	1 : 4
70	2	10	12	1 : 5
139	2	9	11	1 : 4.5
209	2	6	8	1 : 3
282	2	11	13	1 : 5.5
435	2	22	24	1 : 11
574	7	28	35	1 : 4
692	15	21	36	1 : 1.4
934	25	16	41	1 : 0.64
1156	26	22	48	1 : 0.84
Station 1169. 16° 22′ N-Br., 41° 10′ W-Lg.				
0	2	18	20	1 : 9
50	2	21	23	1 : 10.5
100	2	23	25	1 : 11.5
200	7	22	29	1 : 3.1
300	15	18	33	1 : 1.2
400	30	11	41	1 : 0.37
600	35	12	47	1 : 0.34
790	46	4	50	1 : 0.09
993	50	1	51	1 : 0.02
Station 1172. 11° 43′ N-Br., 40° 54′ W-Lg.				
0	0	20	20	1 : 204
38	0	19	19	1 : 194
76	0	16	16	1 : 164
150	17	17	34	1 : 1
223	31	9	40	1 : 0.3
294	31	13	44	1 : 0.4
136	34	11	45	1 : 0.3
571	41	4	45	1 : 0.1
662	43	2	45	1 : 0.05
838	40	6	46	1 : 0.15
1020	40	5	45	1 : 0.13
Station 1210. 22° 14′ N-Br., 67° 50′ W-Lg.				
0	2	9	11	1 : 4.5
50	2	12	14	1 : 6
100	2	12	14	1 : 6
200	4	10	14	1 : 2.5
300	6	9	15	1 : 1.5
400	9	8	17	1 : 0.9
600	21	8	29	1 : 0.4
800	33	7	40	1 : 0.2
1000	36	5	41	1 : 0.14
Station 1217. 29° 55′ N-Br., 65° 15′ W-Lg.				
0	4	11	15	1 : 2.8
39	3	—	—	—
78	3	11	14	1 : 3.7
155	4	12	16	1 : 3
233	4	10	14	1 : 2.5
312	5	9	14	1 : 1.8
471	9	6	15	1 : 0.7
633	16	12	28	1 : 0.75
817	20	17	37	1 : 0.85
1166	26	16	42	1 : 0.6
Station 1222. 33° 15′ N-Br., 67° 35′ W-Lg.				
0	1	14	15	1 : 14
39	2	–	—	—
77	2	10	12	1 : 5
155	2	14	16	1 : 7
232	1	14	15	1 : 14
310	2	12	14	1 : 6
470	2	15	17	1 : 7.5
633	8	13	21	1 : 1.6
775	12	10	22	1 : 0.9
1035	22	14	36	1 : 0.64

Andererseits befindet sich das Wasser keineswegs in Ruhe, und horizontale Wasserbewegungen können das Verhältnis Phosphatphosphor : organischer Phosphor verschieben. Ein derartiger Fall, in dem organischer Phosphor durch horizontale Ströme angereichert ist, mag auf den Stationen 1154 und 1217 in der Tiefe vorliegen, auf denen der organische Phosphor seine höchste Konzentration im unteren Teil der 1000 m-Schicht erreicht.

Das Verhältnis Phosphatphosphor : organischer Phosphor schwankt von mehr als 1 : 20 bis weniger als 1 : 0.02, wobei das größere Verhältnis für den oberen Teil der Wassersäule und das kleinere Verhältnis für den unteren Teil typisch ist. Die vertikale Verteilung des Verhältnisses Phosphat : organischer Phosphat ist im Prinzip das Ergebnis der vertikalen Verteilung des Phosphats selbst.

In der vorhergehenden Besprechung der Verhältnisse in den Oberflächenschichten schlossen wir versuchsweise auf Grund der Werte für organischen Phosphor, daß die äquatoriale Zone produktionsfähiger als das Sargassoseegebiet ist. Eine Bestätigung findet diese Ansicht, wenn man die vertikale Verteilung des Phosphats auf den Stationen 1210, 1217, 1222 und 1154 mit der Verteilung auf Station 1172 vergleicht. In der Sargassosee schwankt die Dicke der phosphatarmen Schicht (0—5 mg P/m³) zwischen 250 und 625 m mit einem Gehalt an organischem Phosphor, der auch in der Produktionsschicht 12 mg/m³ nicht überschreitet. Mit der Annäherung an den Äquator wird die phosphatarme Schicht

dünner. Auf Station 1169 reicht die Schicht mit 0—5 mg/m³ anorganischen Phosphor bis zu einer Tiefe von 175 m mit einem Gehalt von 26 mg/m³ organischen Phosphor in 100 m Tiefe. Auf Station 1172 ist die phosphatarme Schicht weniger als 125 m dick bei einem Gehalt von 20 mg/m³ organischen Phosphor im Oberflächenwasser. Der Unterschied in der Produktionsfähigkeit der beiden Gebiete ist demnach offenbar eine Tatsache, die nicht nur durch den Gehalt des Oberflächenwassers in anorganischen Phosphor, sondern auch durch die Dicke der nährstoffarmen Wasserschicht gekennzeichnet wird.

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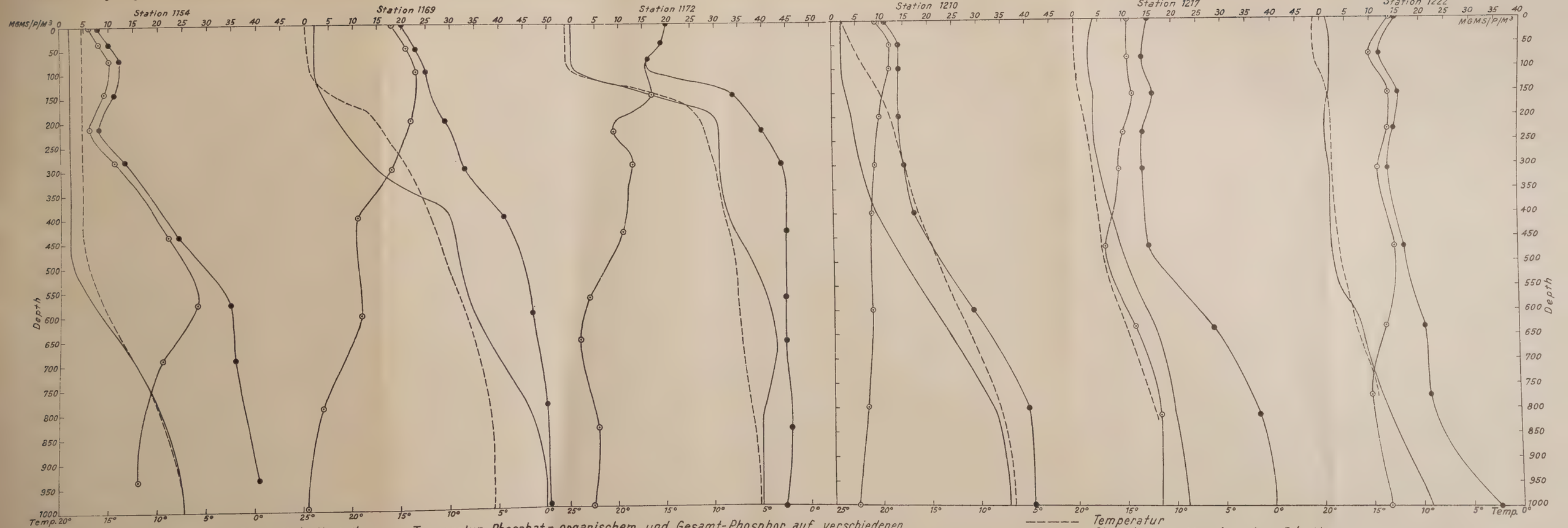
H.R. Seiwell und G.E. Seiwell

Über den Gesamtphosphorgehalt des Seewassers im westlichen Nordatlantischen Ozean



Die vertikale Verteilung von Temperatur, Phosphat,- organischem und Gesamt-Phosphor auf verschiedenen Stationen im nordwestlichen Atlantischen Ozean. Betreffs der Positionen vergleiche Text-Figur u. Tabelle 2.

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A METHOD FOR THE DETERMINATION OF AMMONIA IN WATER AND AIR

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In the course of a study of the organic material present in solution in sea water, it became necessary to measure also the quantity of nitrogen present as ammonia, and, since the method worked out for this purpose appears to be of wider applicability, especially in oceanography, I propose to describe the method finally arrived at in some detail. It can, with a little practice, be carried out fairly easily and very rapidly even on board ship, a condition which appears to be absolutely essential, since no treatment has been discovered so far which will keep the ammonia of sea water samples at the original level.

The principle of the method is to make a sample of water containing from 0.05 to 2 γ (gamma = 0.001 mg.) ammonia nitrogen (generally 20 ml.) just alkaline, to distill off in a partial vacuum the ammonia together with about 5 ml. of the water into a small volume of weak acid in which the ammonia can be titrated with naphthyl red according to the method of Teorell (1932).

The Distillation

The following reagents are required:

1. Re-distilled ammonia-free water is prepared from tap water or distilled water containing about 1.5 grams permanganate and 0.1 ml. sulphuric acid per liter by distillation from a Pyrex flask of 2–3 liters capacity. A very effective trap for catching drops is essential and the permanganate is useful mainly because its presence in the trap shows how far drops are carried. About 5 grams of pumice are added to insure quiet boiling. The middle portion of the distillate is taken up in 1-liter glass-stoppered Pyrex bottles and, as a measure of safety, a current of CO_2- and NH_3-free air is blown into the bottle during the distillation. Water for the day's use is poured from the storage bottle into a glass-stoppered bottle of suitable size. This water is used for rinsing and for the preparation of certain further reagents and control samples.

[1] Contribution No. 28.

2. About .25N sodium hydroxide, prepared by dissolving 1 gram of NaOH in 100 ml. NH_3-free water, is used for bringing the water samples to a hydrogen-ion concentration between pH 9 and 11. A sample of the water (20 ml. or a suitable fraction) is titrated in a white porcelain basin with the alkali from a 1- or 2-ml. burette, using cresolphthalein as an indicator. The corresponding amount of alkali or a little more is added to the water sample just before distillation. I have found that even an excess of 50 per cent alkali does not liberate any excess ammonia from the waters so far tested.

3. The ammonia distilled off is taken up in 1 ml. hydrobromic acid about N/100 prepared by adding 0.2 ml. 25 per cent HBr to 133 ml. ammonia-free water. This solution must remain NH_3-free, and if the air in the laboratory shows traces of ammonia it should be protected from contact. Neither the concentration nor the quantity of hydrobromic acid measured off need be determined with any particular accuracy.

The distillation apparatus is constructed upon the general lines laid down by Parnas and Wagner (1921), but certain modifications have been introduced, the most important of which is the use of air at a reduced pressure instead of steam for driving off the ammonia. The distillation flask (Fig. 1, 1) is of Pyrex glass and holds at least 50 ml. in the bulb. It is provided with a standard ground joint of 14–11 mm. Slightly alkaline glycerine is used to lubricate this joint. At least two and preferably three of these flasks should be available for rapid serial determinations.

The connecting piece (2) must also be Pyrex, but the rest of the apparatus can be made from ordinary soda glass. The final 2–3 cm. of the tube (3) should be capillary tubing of 0.5–0.6 mm. diameter constricted at the tip to about 0.1 mm. The part below the condenser can be conveniently constructed from a gas-washing bottle. The bottom is replaced by a rubber stopper through which a glass rod can be pushed up and down to place the collecting bottle (4) at just the right height. For serial determinations a suitable number of these bottles should be available. A metal ring (5) is sealed on to the inside of the bottle with DeKhotinsky cement to prevent the stopper from being sucked in by the vacuum. The stopper and glass rod are kept well lubricated with alkaline glycerine. The 3-way tap (6) connects this part of the apparatus with a vacuum or effective filter pump. Ammonia-free air is admitted to the apparatus through the sulphuric acid bottles (7) and (8). The tube (9) is between 60 and 100 cm. in length and about 0.1 mm. bore and is permanently open. It should provide just enough air for driving over the ammonia when the vacuum is on. The tube (10) is wider. The screw clip (11) is opened only when the distillation is

finished and the pressure raised to that of the atmosphere. When doing a series of distillations, I have always found it necessary to begin with one or more blanks to get rid of traces of NH_3 which will collect even in the closed apparatus. The collecting bottle is charged with 1 ml. N/100 HBr and put in position so that the tip of the tube (3) will just dip into the fluid when the vacuum is put on. About 20 ml. ammonia-free water is put into a distillation flask with a single drop of N/4 NaOH. The evacuation is started as soon as the distillation flask is put on and the burners (11) and (12) ignited. The burner (11) is regulated so as to produce a suitable rate of distillation, usually about 1 ml. per minute. The burner (12) prevents condensation in (2). The bottle (4) is lowered gradually as distillation proceeds so as to minimize splashing, and before it is finished the tube (3) should be clear of the fluid. After distillation of about 5 ml., the gas is turned off, the tap (6) turned so as to shut off the apparatus from the pump without opening it to the atmosphere, and the screw clip (11) opened so as to admit a fairly rapid current of air. It is important to have the gas turned off between determinations as it usually contains appreciable amounts of ammonia. Finally the bottle (4) is taken out and immediately stoppered.

During the distillation of one sample there is time to prepare a fresh collecting bottle and the next sample for distillation. The alkali is added to the latter in the interval during which the pressure in the apparatus is being brought back to the atmospheric level. Following this procedure five determinations can be made in one hour.

The Ammonia Titration

This process has been worked out according to the excellent method introduced by Teorell (1932). Only minor alterations have been made to deal with quantities even smaller than his. The principle of the method is to oxidize the ammonia with a surplus of sodium hypobromite in alkaline solution. After acidification the excess hypobromite is titrated with an acid solution of naphthyl red which becomes decolorized so long as NaOBr is still present.

The necessary reagents are:

1. Sodium hypobromite about n/1,000, made up by dilution from

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Fig. 1. Apparatus for the determination of pre-formed ammonia in water and air.

(1) Distillation flask. (2) Connecting head. (3) Condenser. (4) Receiver. (5) Receiver-holder. (6) Three-way tap connecting with vacuum. (7) and (8) Sulphuric acid wash bottles. (9) Fine capillary for constant admission of air. (10) Clamp for returning to atmospheric pressure. (12) *-a-*, *-b-*, micro-burners. (13) Bottle with syringe-pipette for hypobromite solution. (14) Hydrobromite acid. (15) Absorber for the determination of ammonia in air.

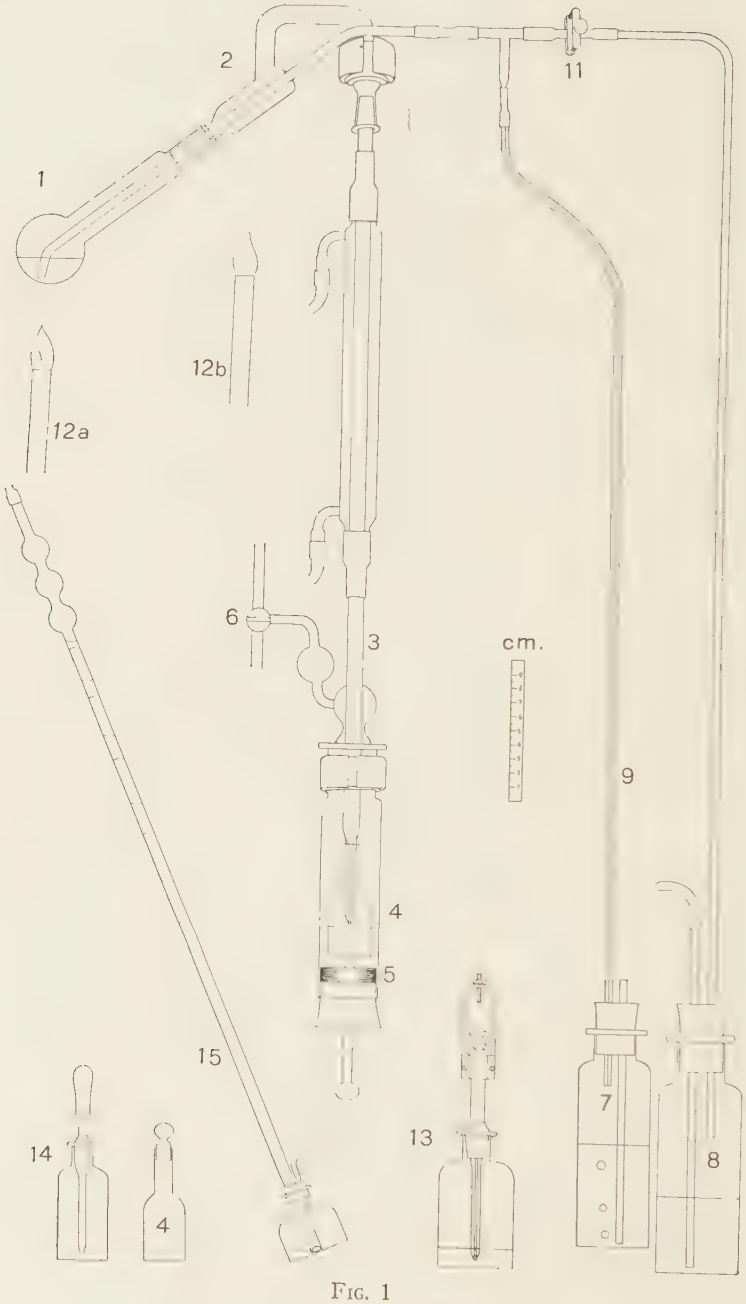

FIG. 1

n/10 stock solution (2.5 grams NaOH, 1.25 ml. fluid bromine in water to 500 ml.). The reaction between NaOBr and NH_3 must take place at a pH of 8.5–9 and the n/1,000 sodium hypobromite is therefore made sufficiently alkaline with 2n Na_2CO_3 to give this pH when about 1 ml. is added to 1 ml. of n/100 HBr. I have found 5 ml. of 2N Na_2CO_3 per 500 ml. suitable.

The stock solution will remain constant indefinitely when kept at a low temperature. The dilution, which must be kept in a dark brown bottle, is not quite constant, but can be used for weeks. I have found it convenient to transfer the quantity for each day's use into a smaller brown bottle and to keep the stock in complete darkness.

2. Hydrobromic acid about 30 cc. 25 per cent diluted with ammonia-free water to 200 cc.

3. Naphthyl red n/2,000 made up by mixing in the order indicated 30 cc. glacial acetic acid, 3.75 cc. 89 per cent H_3PO_4, 1.9 cc. 25 per cent HBr, 10.5 cc. of a freshly prepared 0.1 per cent solution of naphthyl red in water, water to 500 cc.

This solution keeps well, but a small amount of a brown precipitate which interferes with the titration is often formed, and the best plan is to filter off each day the quantity necessary for the day's use.

4. Ammonium sulphate. The stock solution contains 0.4716 gram $(NH_4)_2SO_4$ in 1 liter. This is kept protected against evaporation.

One milliliter of this diluted to 100 ml. with NH_3-free water will contain 1 γ nitrogen per milliliter.

It is convenient to measure off the hypobromite for each titration with a syringe pipette mounted in the bottle as shown in Fig. 1 (13). The volume of the pipette need not be just 1 ml., but is regulated so as to correspond to something between 1.8 and 2.0 ml. naphthyl red. The hydrobromic acid I keep in a small bottle (14) with a ground-in rubber-capped pipette which will deliver approximately 0.2 ml.

The titration is made from a 2-ml. burette divided in 200 parts. It is necessary to do each day 2 to 4 blanks and 2 to 4 titrations of a known amount of ammonia. The results depend to some extent upon the temperature, and when the room temperature undergoes considerable variation more constant results are obtained if the solutions to be titrated are brought to an approximately constant temperature in a water basin.

To do a blank, measure off in the titrating bottle 1 ml. n/100 HBr, add about 5 ml. NH_3-free water, bring to the desired temperature, add hypobromite and shake gently; fill up the burette with naphthyl red to the zero point. This takes about the half minute required for the reaction between hypobromite and ammonia to become complete. In order to secure uniform results constant timing should be adhered to. To the sample is now added 0.2 ml. 4 per cent hydrobromic acid; it is again

shaken up and titrated from the burette to a just visible rose color. It is advisable to use a comparison bottle with water and just enough naphthyl red to produce this color. The titration must not be made too rapidly, but should take at least 1 minute. For the determination of the NH_3 equivalent of the naphthyl red, 1 or 2 ml. of the ammonium sulphate solution is added to the n/100 HBr and enough NH_3-free water to bring the volume to 5–6 ml. The mixture is treated as above and titrated. To test the absorption of NH_3 during distillation, ammonium sulphate is added to 20 ml. water and distilled off.

Example:

	ml. naphthyl red solution
Blanks titrated directly	1.96, 1.95
Blanks distilled	1.95, 1.95
Ammonium sulphate (2 γ N) directly	0.89
Ammonium sulphate (2 γ N) distilled	0.89

2 γ N = 1.06 ml. naphthyl red solution

For the determination of ammonia in fluids where less than 1 γ is regularly to be expected, it may be worthwhile to reduce the volume of hypobromite to .5 ml. The accuracy to be obtained in the single determinations of a series is of the order 0.04 γ N which corresponds by the distillation of 20 ml. to $\pm$ 2 mg. ammonia nitrogen per cubic meter.

The Determination of Ammonia in Air

An apparatus like the one shown (Fig. 1, 15) is suitable. The glass tube is 6 mm. in internal diameter and about 50 cm. long up to the lower of the three bulbs. The top is connected to a water bottle of suitable capacity (4 to 10 liters) from which the water is allowed to run out at a rate of 4 liters per hour or less. The bottle is charged with 1 ml. N/100 HBr and 4–6 ml. NH_3-free water and the whole is placed in a slanting position so that the absorbing fluid moves fairly regularly right up to the bulbs, thus providing a constantly renewed absorbing surface of about 100 cm.^{2} Titration is performed as above in the absorbing bottle. The air in a laboratory for ammonia determination should contain less than 0.1 γ N per liter.

Summary

A method and apparatus for the determination of ammonia in water are described. The ammonia is carried over by a simple vacuum distillation into hydrobromic acid in which it is determined by the naphthyl red titration of added hypobromite.

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ON THE PROPORTIONS OF ORGANIC DERIVATIVES IN SEA WATER AND THEIR RELATION TO THE COMPOSITION OF PLANKTON[1]

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> "Chemical analysis shows that the animal and plant body is mainly built up from the four elements, nitrogen, carbon, hydrogen, and oxygen. Added to these are the metals, sodium, potassium and iron, and the non-metals, chlorine, sulphur and phosphorus. Calcium or silicon are also invariably present as the bases of calcareous or siliceous skeletons. All these, with some others, are indispensable constituents of the organic body, and in an exhaustive study of the cycle of matter from the living to the non-living phases, and *vice versa*, we should have to trace the course of each." JAMES JOHNSTONE, "Conditions of Life in the Sea," p. 273. 1908.

IT is now well recognized that the growth of plankton in the surface layers of the sea is limited in part by the quantities of phosphate and nitrate available for their use and that the changes in the relative quantities of certain substances in sea water are determined in their relative proportions by biological activity. When it is considered that the synthetic processes leading to the development of organic matter are limited to the surface layers of the sea in which photosynthesis can take place, it becomes evident that the chemical changes which occur in the water below this zone must arise chiefly from the disintegration of organic matter. In so far as this disintegration goes to completion, the changes in the derived inorganic constituents of sea water must depend strictly upon the quantity and composition of the organic matter which is being decomposed. This is true quite irrespective of the agencies of decomposition, be they bacterial action,

1. Contribution No. 30, from the Woods Hole Oceanographic Institution.

the autolytic enzymes of the original tissues themselves, or the metabolic processes of deep sea animals which utilize the organic matter as food. Thus, in the decomposition of a given quantity of organic matter, the quantity of oxygen consumed must be determined by the quantity of carbon, nitrogen, hydrogen, sulphur, and phosphorus to be oxidized, and the relative changes in the quantity of oxygen, nitrate, phosphate, carbonate, and sulfate must depend exactly on the elementary composition of the plankton.

It has seemed possible that the chemical composition of the population of the seas may be sufficiently uniform, and the contributions of the substances in question from other sources sufficiently limited, to permit the discovery of relations between their concentration in sea water which would be serviceable in the study of oceanic problems.

An examination of the data on nitrate and phosphate in the water of various seas secured by the " Dana " in the course of the Carlsberg Foundation's oceanographical expeditions around the world in 1928-1929 proved encouraging from this point of view. Thanks to the collaboration of Dr. Norris Rakestraw a more complete set of data, showing the relative concentrations of oxygen, nitrate, phosphate, and carbonate at different depths, was obtained from a number of stations made by the " Atlantis " in the deep waters of the western Atlantic Ocean. Six stations were occupied in the Sargasso Sea between Bermuda and the southern coast of the United States. In this region water samples were obtained at successive depths from at least three distinct water masses : (1) the surface water in intimate relation with the atmosphere and in which photosynthetic activity has reduced the nutrient substances to a minimum ; (2) the layer of intermediate depth—most clearly marked at 700 meters and characterized by minimal oxygen concentrations and high concentrations in organic derivatives ; and (3) the deep water of polar origin of relatively uniform composition below 1,500 meters, which has also been the seat of organic decomposition. Between these layers lie zones in which the water has intermediate characters due to the mixing of the primary water masses.

The characteristics of the water at the various stations occupied are unusually uniform both as regards temperature and salinity and the concentrations of the biologically significant substances. A seventh station was occupied in the western edge of the Gulf Stream outside the Gulf of Maine. Here the layer characterized by minimal oxygen concentration is nearer the surface and the deep cold water lies within 500 meters of the surface. The numbers and positions of the stations are given in Table I. The station data will be published in the Bulletin Hydrographique of the International Council for the Exploration of the Sea.

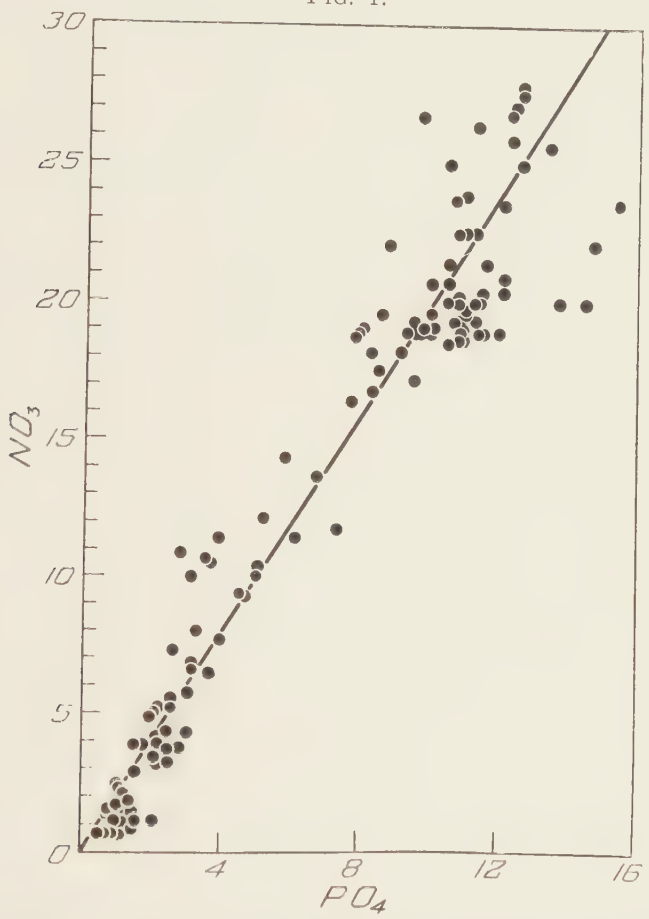
TABLE I

Ship	Station No.	Position	Date
" Atlantis "	1463	34°02′ N. 68°15′ W.	February 8, 1933
„	1465	31°25′ N. 66°30′ W.	„ 13, 1933
„	1467	30°30′ N. 68°30′ W.	„ 14, 1933
„	1469	29°26′ N. 70°42′ W.	„ 15, 1933
„	1472	27°56′ N. 73°53′ W.	„ 17, 1933
„	1475	26°49′ N. 76°09′ W.	„ 19, 1933
„	1677	40°53′ N. 66°21′ W.	June 25, 1933

From the data for oxygen content and temperature, the amount of oxygen which has disappeared from the water since its exposure to the atmosphere has been estimated, assuming that the water was saturated at that time and had the temperature which characterized it *in situ*. While neither of these assumptions are probably strictly correct, they provide the only definite method available to arrive at the amount of oxygen utilized at the various depths. From the data for pH as measured at the temperature of the ship's laboratory the total carbonate content, Σco_2, of the water samples was estimated with the aid of the tables provided by Buch, Harvey, Wattenberg, and Gripenberg (1932). These,

together with the direct analyses of nitrate and phosphate, give a measure of the principal products formed by the complete oxidation of the carbon, nitrogen, and phosphorus of the biological population, together with the amount of dissolved oxygen which has disappeared in the process.

FIG. 1.



Correlation between concentrations of nitrate and phosphate in the waters of western Atlantic Ocean. Ordinate, concentration of nitrate, units 10^{-3} millimols per liter; abscissa, concentration of phosphate, units 10^{-4} millimols per liter. The line represents a ratio of $\triangle N : \triangle P = 20 : 1$ milligram atoms.

The data obtained for the water masses underlying the various stations are plotted in Figs. 1, 2 and 3. In these diagrams the concentration of nitrate characterizing each

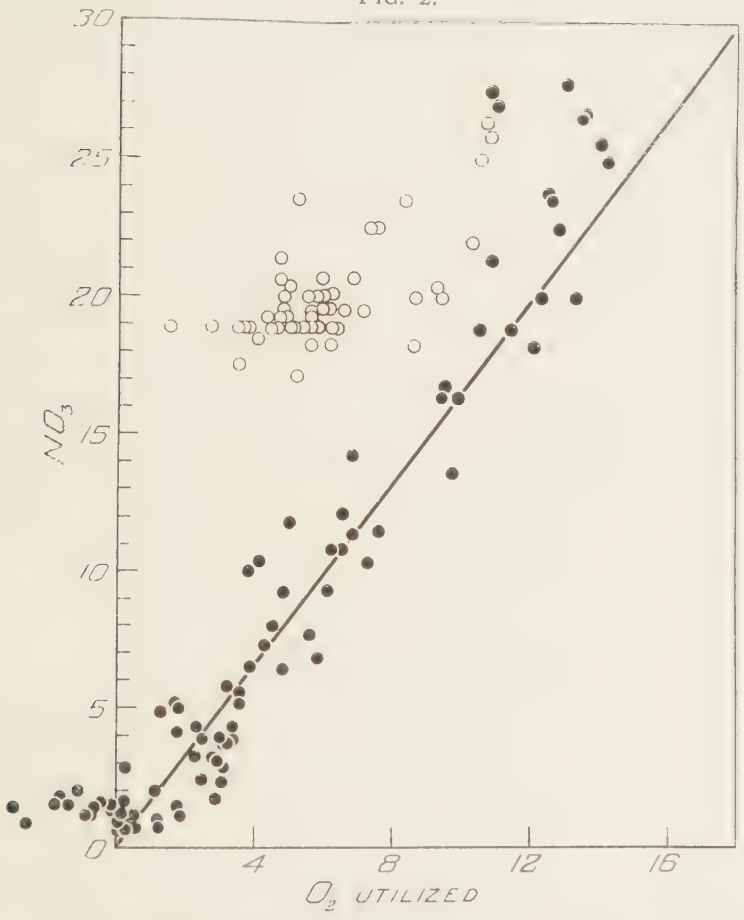
water sample is plotted against one of the other constituents in order to show whether the concentrations of these constituents vary in a correlated fashion. The concentrations are expressed in milligram atoms or in millimols per liter, as suggested by Cooper (1933), since this notation yields values proportional to the number of atoms of nitrogen, carbon, and phosphorus present in the constituents in question.

To consider first the relation between nitrate and phosphate concentration in the water samples, shown in Fig. 1, it is apparent that a very close correlation exists. Any gain or loss in nitrate content observed in comparing one water sample with another is accompanied by a strictly proportional gain or loss in phosphate content. The water samples in which the concentrations were low were from near the surface; the quantities of nitrate and phosphate increase with increasing depth. The straight line drawn through the points describing the nitrate-phosphate correlation has the slope demanded if, for every three grams of nitrogen added to or subtracted from the water, one gram of PO_4 (or $\frac{3}{4}$ gram of P_2O_5) also made its appearance or disappearance. This corresponds to a ratio of 20 atoms of nitrogen to 1 atom of phosphorus. On the assumption that these changes are due solely to the decomposition or synthesis of organic matter, these ratios may be taken to reflect the proportions of nitrogen and phosphorus in the plankton community taken as a whole.

The correlation between the gain in concentration of nitrate and the loss in concentration of oxygen, as shown in Fig. 2, is almost equally good for the water samples collected from depths less than 1,000 meters in the Sargasso Sea, and 400 meters in the Gulf Stream,—depths which mark the transition between the intermediate layer of low oxygen content and the deeper layer of cold water. The data for greater depths, indicated by open circles in the figure, depart strongly from the expected correlation—an anomaly to which we will return. Above these depths, however, the variation of nitrate concentration and oxygen utilization is in the proportion of 1 millimol of nitrate to 6 millimols of oxygen.

The correlation between the concentrations of nitrate and carbonate shown in Fig. 3 is less precise owing to the greater variation in the carbonate measurements. This is due to the fact that the total variation in carbonate content is

FIG. 2.

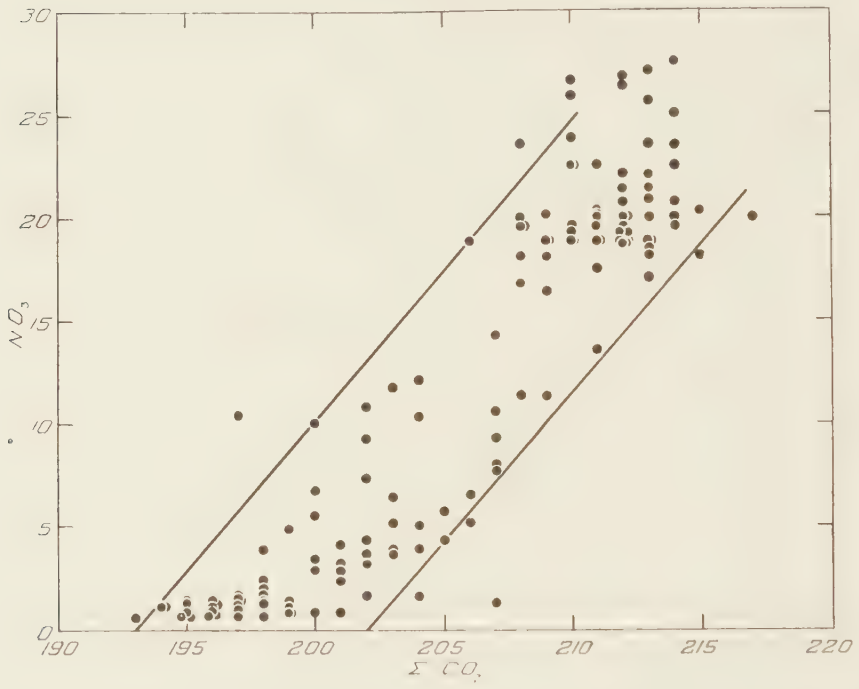


Correlation between concentration of nitrate and amount of oxygen utilized in waters of western Atlantic Ocean. Ordinate, concentration of nitrate, units 10^{-5} millimols per liter. The line represents a ratio of oxygen used : $\triangle N = 6 : 1$. Points representing stations from a depth below 400 meters in the Gulf Stream and below 1,000 meters in the Sargasso Sea are represented by open circles.

only a small part of the amount present, to the indirect nature of the method of estimating carbonate, to possible variations in buffer capacity which have been neglected in these

estimations, and to exchange in CO_2 between air and the surface waters. Nevertheless, taking the data as a whole, it is clear that carbonate content is correlated with nitrate content. The envelopes drawn about the points in Fig. 3 have a slope corresponding to a ratio of 1 millimol of nitrate to 7 of carbonate. The value of this ratio is not as securely established as that relating nitrate to phosphate and to oxygen utilization.

FIG. 3.



Correlation between concentration of nitrate and total carbonate in waters of western Atlantic Ocean. Ordinate, concentration of nitrate, units 10^{-3} millimols per liter; abscissa, concentration of carbonate, units 10^{-2} millimols per liter. The slope of the envelopes corresponds to a ratio of $\triangle C : \triangle N = 7 : 1$ milligram atoms.

If we take the ratio of change in nitrate to carbonate to be 7 : 1 and that of nitrate to oxygen utilization to be 6 : 1, it follows that the ratio of carbonate to oxygen is 7 : 6 or 1·17 : 1. This ratio is close to that characterizing photosynthetic processes and the decomposition of the principal classes of

organic compounds. Its variation from the theoretical is not greater than the uncertainty of the carbonate measurements.

It appears from the foregoing data that, with the exception of the anomaly quoted in connection with the oxygen content of the deep water, the concentrations of nitrate, phosphate, and carbonate in samples of oceanic sea water of widely different origin vary in such a way as might be expected if the different samples contained the products of the complete disintegration and oxidation of organic material of similar composition, and that they differ only in the quantity of such material which has arrived at and remains in this degree of decomposition. Furthermore, the loss in oxygen agrees approximately with that to be expected from the quantity of carbonate gained. The proportions of carbon, nitrogen and phosphorus present in the organic material from which the carbonate, nitrate, and phosphate may be supposed to be derived are approximately 140 : 20 : 1 atoms or 100 : 16·7 : 1·85 grams.[1]

It is pertinent to inquire how these proportions agree with those actually found in various members of the plankton community. Naturally each kind of organism is found to have a characteristic composition different from that of other kinds, and unfortunately no adequate means of obtaining a truly representative sample of the entire population is available. However, by considering the composition of the plankton yielded by various kinds of haul, some idea may be obtained as to the validity of the foregoing considerations.

A number of elementary analyses of various kinds of plankton are recorded in Table II. They show that the proportions of carbon, nitrogen, and phosphorus as calculated from the carbonate, nitrate, and phosphate composition of the sea are not greatly different from those observed in

1. This comparison presupposes that nitrate nitrogen may be regarded as the sole source of nitrogen available to the plankton. Strictly speaking, this is not the case. Of other sources of nitrogen, nitrite is known to occur in oceanic waters in concentrations too small to be significant in computations of this sort. Professor Krogh has recently made observations on the nitrogen present as ammonia and as organic compounds in the deeper waters of the western Atlantic. His results indicate that ammonia is present in only small quantities. Organic compounds in the sea water contain relatively large amounts of nitrogen, but as the concentration of these does not vary greatly from surface to bottom, this source of nitrogen does not appear to be readily available for conversion into living matter.

various plankton, and on the whole the latter differ among themselves much more than their average differs from the calculated ratios.

TABLE II

Proportions of carbon, nitrogen, and phosphorus in various samples of plankton.

Sample	Parts by Weight		
	Carbon	Nitrogen	Phosphorus
Mixed copepods from Buzzards Bay ..	100	21	1·98
Centropages typicus, Gulf of Maine ..	100	25·6	1·06
Calanus finmarchicus, Gulf of Maine ..	100	13·4	2·04
Calanus finmarchicus, Gulf of Maine ..	100	15·8	2·26
Diatoms—Bay of Fundy, almost entirely *Thalassiosira nordenskiöldi*.	100	18·2	1·36
Diatoms—Off Nova Scotia coast—17 species of somewhat the same abundance.	100	15·6	2·26
Peridinians—Meyer (1914)	100	13·2	2·2
Chiefly peridinians—average of samples No. 1, 2, 3, 4, of Brandt (1898).	100	8·1	—
Chiefly diatoms—average of samples No. 6 and 7, Brandt (1898).	100	12·4	—
Chiefly copepods—average of samples No. 8 and 9, Brandt (1898).	100	15·3	—
Mixed plankton—sample no. 10, Brandt (1898). ..	100	11·3	—
Average all samples	100	15·4	1·88
Estimated from analyses of sea water ..	100	16·7	1·85

In this connection it is of interest to note that Braarud and Føyn (1930) have observed that each cell of *Chlamydomonas* removes $2{\cdot}98 \times 10^{-12}$ gr. NO_3 nitrogen and $0{\cdot}98 \times 10^{-12}$ gr. P_2O_5 from the sea water in which it grows. Here we see in a laboratory experiment an organism modifying the concentration of nitrate and phosphate in the medium in a ratio ($\triangle N : \triangle P = 15 : 1$) not very different from that observed in the oceans as a whole.

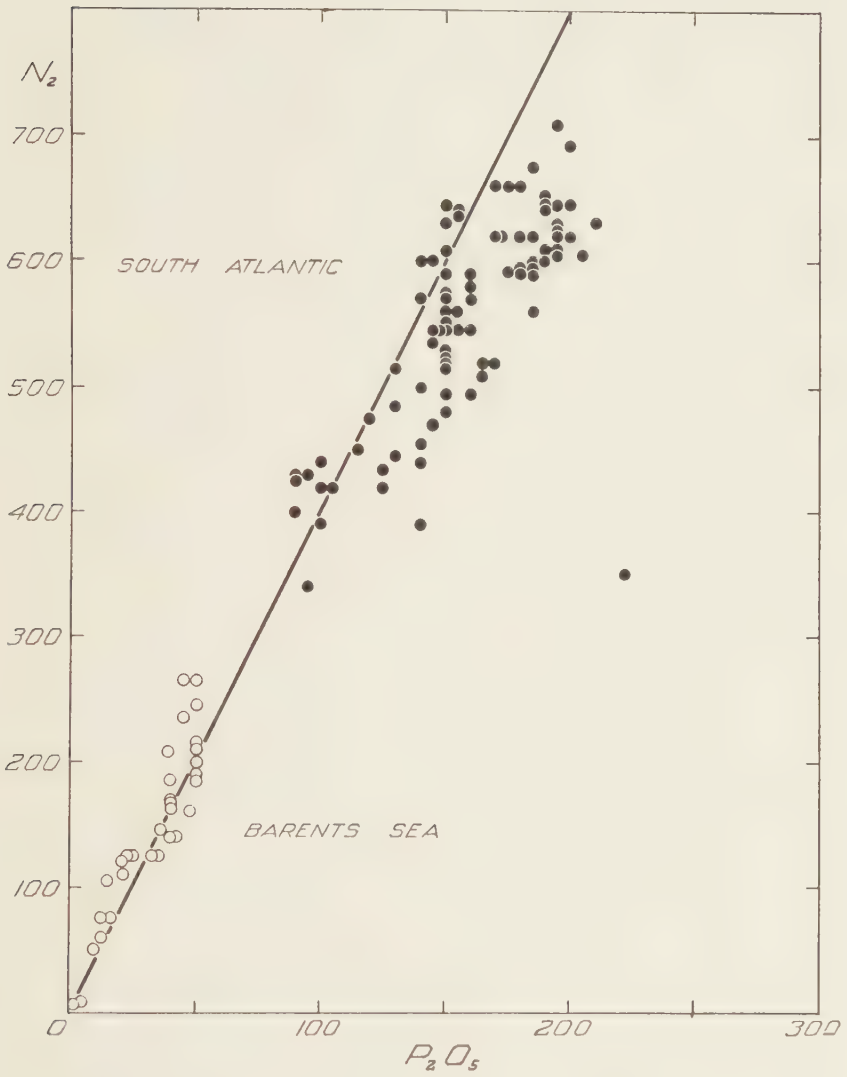
PACIFIC
INDIAN
ATLANTIC
N_2
500

The establishment of a general, if approximate, relation between the concentration of the various organic derivatives in sea water and the chemical composition of plankton would provide a valuable tool in the analysis of many oceanographic problems. Observations made at a single group of stations such as that reported cannot be considered to establish such a relation, though the results doubtless are encouraging. The data collected by the "Dana" (Thomsen, H., 1931) enable the generalization to be tested more widely—at least so far as nitrate and phosphate are concerned, for the measurements were made in the most diverse regions by the same investigators using uniform methods. In Fig. 4 I have plotted the simultaneous measurements of phosphate and nitrate obtained by the "Dana" at all stations and depths in the three oceans traversed. No attempt has been made to separate the data obtained at different stations within each ocean, for, as in the case of the observations collected by the "Atlantis," the water underlying a given station at various depths has quite different origins. It is apparent that a definite correlation exists between the quantity of nitrate and phosphate occurring in any sample. The proportions between these constituents, as indicated by the lines drawn through the points, are essentially the same in the three oceans visited by the "Dana," and in the Atlantic stations occupied by the "Atlantis".

The stations occupied by the "Dana" and the "Atlantis" were all in temperate or tropical latitudes. In Fig. 5 are plotted data selected at random from the stations in Barents Sea, 70°-76° N, reported by Kreps and Verjbinskaya (1932) and from stations in the south Atlantic Ocean between 55° and 62° S. published by Ruud (1930). Here the proportions between phosphate and nitrate nitrogen vary in the same ratio as in the more temperate waters found at low latitudes, though the quantities of these constituents are much lower in the north than in the south.

These results indicate that the relations observed in the western Atlantic may be of general application, at least so far as the oceanic waters are concerned. By inference they

FIG. 5.



Correlation between concentration of nitrate and phosphate in waters of Barents Sea (data of Kreps and Verjbinskaya) and south Atlantic (data of Ruud, from stations between 55°-62° S., 0°-40° W.). Ordinate, concentration of nitrate nitrogen, units milligrams of nitrogen per cubic meter, abscissa, concentration of phosphate, units milligrams P_2O_5 per cubic meter. The line corresponds to a ratio of $\triangle$ N : $\triangle$ P = 20 : 1 milligram atoms.

suggest a remarkable uniformity in the chemical composition of the planktonic communities occupying the various oceans. It is scarcely to be expected that these relations will hold in water bodies of limited extent, where local conditions such as the proximity of land, the inflow of rivers, and the peculiarities of a local flora and fauna may alter the picture. Examination of data for such regions as the Baltic (Buch, 1932), the Norwegian fjords (Braarud and Klem, 1931), the Denmark strait (Bohnecke, Føyn, and Wattenberg, 1932), indicate that this is the case. In the English Channel the ratio in the variation in nitrate and phosphate in the surface water with season was about 2 mg. nitrate nitrogen to 1 mg. P_2O_5 ($\triangle N : \triangle P = 10 : 1$ mg. atoms) according to data published by Harvey (1928). Cooper (1933) has shown that in this region where active regeneration takes place, variation in the ratio of nitrate to phosphate is attributable to the longer time required to complete the regeneration of nitrate from decomposing albuminous matter. In the Gulf of Maine data recently secured by Dr. Rakestraw indicate that the ratio of the variation of nitrate and phosphate with depth is lower and more variable than in the open ocean outside the Gulf. However, the value of a generalization such as that suggested by the data from the open oceans lies in the fact that it makes clearer the exact nature of the anomalous conditions found in local situations and thus defines more precisely the questions which must be answered before the local situation is understood. This point can be illustrated by a consideration of the anomaly in the concentration of oxygen which is present in the data secured by the " Atlantis ".

Below 1,000 meters there is less oxygen removed from the water than is to be expected from the amount of nitrate present. This expectation, however, is based on the assumption that the nitrate has all appeared as the result of decomposition of organic matter after the water was removed from contact with the atmosphere. The observations suggest that the deeper water of the Atlantic contained a considerable amount of nitrate at the time it sank. Actually one must assume most of the samples from the greater depths to have

contained about 140 mg. nitrate nitrogen per cubic meter (equivalent to 10×10^{-3} mg. atoms per liter) at the time they became separated from the atmosphere. Now the water at these depths is thought to originate at high latitudes where nutrient substances are commonly observed at high concentrations in the surface water. Thus Kreps and Verjbinskaya (1932) observed nitrate nitrogen concentrations in the surface water of Barents Sea of 128 to 200 mg. per cubic meter in March and April, and Bohnecke, Føyn and Wattenberg (1932) record values of 125 mg. per cubic meter in the mixed water of the polar front east of Greenland. The values of nitrate nitrogen recorded by Ruud (1930) in the surface waters of the Antarctic exceed 600 mg. per cubic meter in many cases. Thus the history of the deep water suggested in explanation of the anomalous character of the ratios in which oxygen enters is confirmed by independent observations.

One fact of great general interest emerges from an examination of the data recorded in Figs. 1 and 4. It may be noted that as the quantities of nitrate and phosphate diminish, as they do as one approaches the surface layers of the sea, they diminish simultaneously in such proportions that no great excess of one element is left when the supply of the other has been exhausted. In 1926 Harvey wrote: "It is a remarkable fact that plant growth should be able to strip sea water of both nitrates and phosphates, and that in the English Channel the store of these nutrient salts, formed during autumn and winter, should be used up at about the same time." The relation noted by Harvey is thus of much wider application than he could have known at that time. It appears to mean that the relative quantities of nitrate and phosphate occurring in the oceans of the world are just those which are required for the composition of the animals and plants which live in the sea. That two compounds of such great importance in the synthesis of living matter are so exactly balanced in the marine environment is a unique fact and one which calls for some explanation, if it is not to be regarded as a mere coincidence. It is as though the seas had been created and populated with animals and plants and

all of the nitrate and phosphate which the water contains had been derived from the decomposition of this original population.

Professor Huntsman has suggested to me that the correspondence between the proportions of phosphate and nitrate in the seas and the statistical composition of living matter may be due to the fact that, although different members of the plankton community have different requirements in regard to phosphorus and nitrogen, the numbers of each of these members may depend on the relative availability of the substances they most need. Thus a population requiring relatively much nitrogen may thrive until the nitrate supply has been depleted, when it will be replaced by a population requiring relatively more phosphorus. By the balancing of such communities the ratio of the elements in the plankton as a whole might come to reflect the ratio of the nutrient substances in sea water rather closely.

Another explanation of the correspondence between the proportions of nitrate and phosphate in the sea and the composition of living matter may be sought in the activities of those bacteria which form nitrogenous compounds from atmospheric nitrogen or liberate nitrogen in the course of the decomposition of organic matter. In the case of the nitrogen-fixing bacterium, *Azotobacter*, it is known that for every unit of nitrogen fixed or assimilated and synthesized into microbial protein, about one half a unit of P_2O_5 must be available. The physiological activity of such organisms must tend to bring the relative proportions of organic nitrogen and phosphorus toward the ratio in which these substances occur in the bacterial protoplasm. Comparable studies upon the physiology of the denitrifying bacteria do not appear to have been made. It is evident, however, that the composition of these organisms must be more or less fixed in regard to their relative phosphorus and nitrogen content and that when living in an environment containing an excess of nitrate, considered in relation to phosphate, the growth and assimilation of the organisms may continually tend to bring the proportions of nitrogen and phosphorus nearer to that

characteristic of their own substances. It would appear inevitable that in a world populated by organisms of these two types the relative proportion of phosphate and nitrate must tend to approach that characteristic of protoplasm in general and that, given time enough and freedom from systematic disturbing influences, a relationship between phosphate and nitrate such as that observed to occur in the sea must inevitably have arisen. On this view the quantity of nitrate in the sea may be regulated by biological agencies and its absolute value determined by the quantity of phosphate present.

These explanations are in no wise mutually exclusive; nor do they exclude other possibilities. Whatever its explanation, the correspondence between the quantities of biologically available nitrogen and phosphorus in the sea and the proportions in which they are utilized by the plankton is a phenomenon of the greatest interest.

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REPORT ON EXPERIMENTAL USE OF A TRIANGULAR TRAWL FOR BATHYPELAGIC COLLECTING*

With an Account of the Fishes Obtained and a Revision of the Family Cetomimidae

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INTRODUCTION AND GENERAL DISCUSSION OF METHOD

During the first Caribbean cruise of the "Atlantis," sponsored jointly by the Woods Hole Oceanographic Institution and by Yale University, several experiments were made to test the practicability and usefulness of making deepsea tows with a large triangular trawl, measuring 50 feet along each side of the opening, a gear of this type having been provided for the purpose by the Bingham Oceanographic Foundation of Yale University.

While special mechanical difficulties in the equipment and arrangements for handling the trawl from the ship impeded the use of this large net under the circumstances under which it was tried, one of the trawling attempts nevertheless followed a normal course and yielded a result, to be reported upon on the following pages, which adequately proves the advantages of using this large gear for bathypelagic collecting to supplement the results obtained from the smaller nets. The obstacles encountered in these first experiences with the three-cornered trawl employed in deepsea towing were all of a nature which could easily be corrected in port but could not be remedied underway, and the great success of the one unobstructed haul made during this single cruise in spite of the difficulties met with on board must therefore be taken as a very promising sign for the future use of the gear.

As already mentioned, the trawl net itself measured 50 feet along each side of the opening, between the otter boards, being hung on a steel wire with manilla seizing of 2½ inch circumference. The total length of the net was 36.9 meters (128 feet), the last 4 meters (13 feet) being a straight cod-end with 8 mm. (5/16 inch) mesh from knot to

* Contribution No. 35, Woods Hole Oceanographic Institution.

knot, the rest of the net being tapered with meshes decreasing from 25 mm. (1 inch) from knot to knot in, roughly, the first 40 feet of the length through 20 mm., 15 mm., and 12 mm. to 10 mm. in the last section before the cod-end. Cotton twine was used throughout, and the net was untanned (by misunderstanding) when the hauls were made. The dimensions of the two bottom otter boards were 200 x 110 x 5 cm. (78 x 43 x 2 inches) with a 78 x 6½ x 2 inch iron keel apart from iron half-rounds around the edges and a heavy pair of hinged rod-iron bridles for attachment of the wire-bridle, giving each trawl board a net weight of 167 kilograms (368 lbs.) when dry. The third (top) otter board was smaller and lighter, without iron weight, measuring 120 x 80 x 3 cm. (47 x 31½ x 1⅜ inch) and weighing only 55 kilograms (121 lbs.). The total weight of the three otter boards in dry condition thus adds up to 857 lbs. and exceeds 1000 lbs. after they have once been used for deepsea towing, and this weight was perhaps the greatest difficulty in handling the gear during the cruise, since a winch-boom was not yet available on the ship. The net was attached to the main towing cable by three bridle ropes 45 meters (147½ feet) long of galvanized steel wire 14 mm. (ab. 9/16 inch) circumference.

The accompanying diagrammatic sketch gives a general idea of the relative dimensions and appearance of trawl and ship.

It is believed that the length of the net might be reduced to about 75 feet, and also that the bottom trawl boards might be made much lighter and probably also somewhat smaller without any loss in fishing efficiency, but with great gains in the ease of handling. This, however, will be a matter for future experimentation, but as a general rule the chief requirements for the convenient handling of a three-cornered trawl of these large dimensions for deep-sea towing from a single cable may be stated to be the following: (1) a trawling boom or gallows well forward at the side of the ship; (2) trawling blocks in the boom and guide blocks on deck for guiding the wire to the winch with sheaves of sufficient capacity to permit the bridle to be paid out gradually and to be brought back on board in one continuous haul without necessitating attachment and detachment from the main cable overboard, i. e., after, respectively before, it has passed the last block in the trawl boom; (3) a winch boom well aft from which the otter boards can be lowered with room for the bridle to spread from the trawl boom sufficiently to permit the net to open enough to keep from tangling before it is completely "set," i. e., before the third otter board is released.

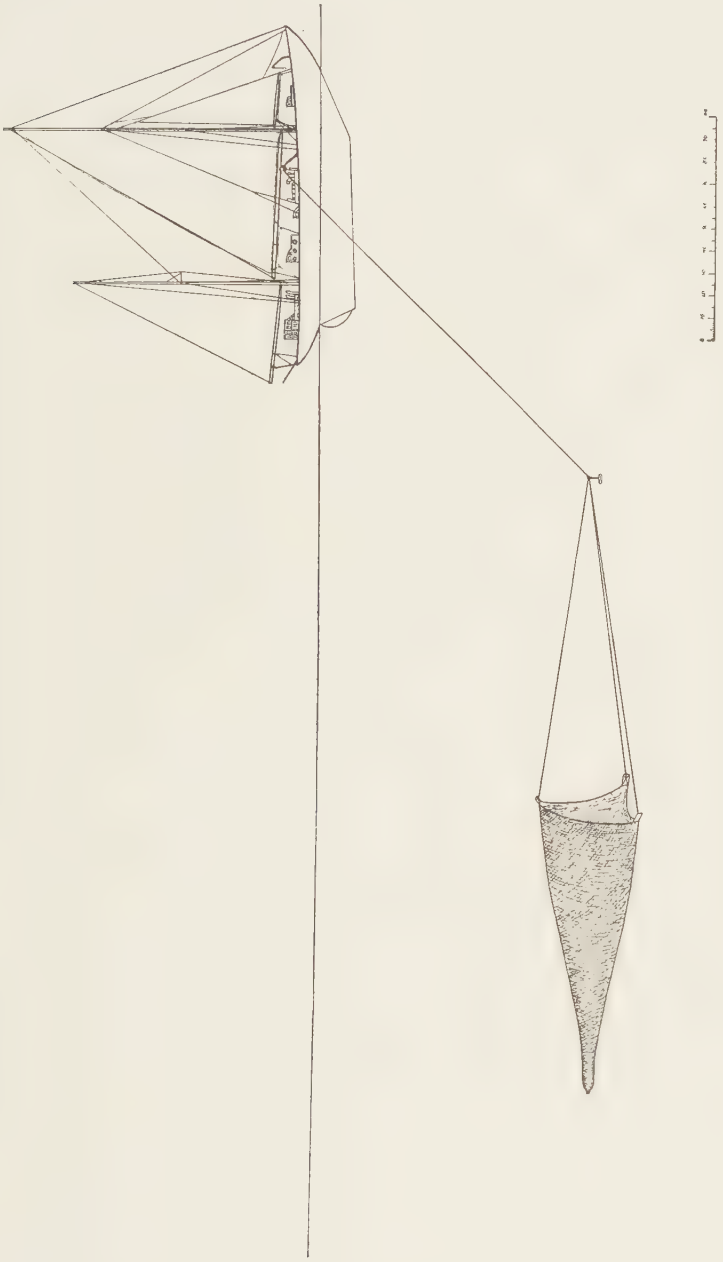

Figure 1. Diagrammatic sketch of research ship Atlantis with 50-foot triangular trawl in tow near surface.

When setting the trawl the ship should be going ahead at slow speed and turning sharply in circles towards the side from which the net is lowered. The bag (cod-end) should first be thrown overboard and permitted to trail out. Thereupon the two bottom otter boards should be lowered and released simultaneously in correct positions from the winch boom aft, with only enough bridle to reach from the trawl boom to the point where they are lowered. After they have spread as much as this length of bridle will permit, the top board should be lowered slowly to the surface and released after the bottom boards have gone down enough to remain clear. This may require that some bridle is paid out simultaneously with the lowering of the top board. After all three boards have thus been released and the net has been observed to be clear, the brakes on the trawl winch should be loosened and the net should be permitted to pull out its own cable, or if power is required, it should be so adjusted that there is always sufficient strain on the cable to keep the net open also when the cable is being paid out.

RESULTS AT ATLANTIS STATION 1478

At Atlantis Station 1478, in 25° 39′ Northern Latitude and 77° 18′ Western Latitude the triangular trawl was lowered to about 1050–1100[1] meters vertical depth and trawling begun at 3:55 P. M. on February 20, 1933. On February 21 at 2:53 A. M. one started heaving in again, and at 5:06 the trawl was at the surface, giving a towing time of about 10 hours in a depth of 1050–1100 meters and a total towing time of about 13 hours including hoisting and lowering.

As the following list will show, this single haul yielded a total of 47 different species of deepsea fish represented by 491 specimens, of which there were 118 specimens of species other than those of the genus *Cyclothone*. For comparison may be mentioned that 39 species was the highest number obtained by the "Pawnee" in 1927 in any one of 18 successful deepsea hauls in Bahaman and Bermudan waters with a ringnet of 14 feet diameter at the opening. If we carry the comparison further, we find an even more striking difference between the number of *large* specimens obtained in the triangular trawl at Station 1478 and the average number of large specimens in the above-mentioned 18 successful[2] hauls with a fourteen-foot ringnet.

[1] 1750 meters wire at 52° angle.

[2] Only the 18 hauls yielding a reasonable result to indicate successful operating

Since the size which constitutes a large specimen of course varies from one systematic group to another, it is naturally also necessary to adopt a variable set of arbitrary standards for the definition of the term large. Thus Iniomi have been considered large when they exceed 100 mm. length without caudal fin, but Apodes and Lyoneri only when they exceed 300 mm. Applying a variable set of standards of this sort in the same manner to both collections, we find the haul from Atlantis Station 1478 containing no less than 22 large specimens while the 18 hauls of the Pawnee give us an average of only 6.78 large specimens each, even when we consider the fact that the average towing time for these 18 hauls was only about half the towing time at Atlantis Station 1478 and accordingly divide the total for the Pawnee trawlings with 9 only, instead of 18. Thus there can hardly be any doubt about the superiority of the large triangular trawl for the capture of the larger specimens and species. If, on the other hand, we compare the total number of specimens of all species, with due consideration of trawling time, the situation is the reverse; which has to do with the fact that the smallest species are the numerically most abundant, and that proportionally fewer of these small individuals will be retained by the larger and of necessity also coarser net. The function of the large triangular trawl can therefore never be to replace entirely the large circular nets now in use but to supplement them for the capture of those larger forms which are too rarely obtained in the smaller gears.

List of Species Obtained in Triangular Trawl at Atlantis Station 1478

ISOSPONDYLI

Stomiatoidea

1 *Bonapartia pedaliota* Goode and Bean. . . . . See page 8.
5 *Gonostoma elongatum* Günther
2 *Gonostoma bathyphilum* Vaillant
3 *Cyclothone braueri* Jespersen and Tåning
112 *Cyclothone pallida* Brauer. . . . . See page 12.
258 *Cyclothone microdon* Günther. . . . . See page 12.
2 *Yarrella blackfordi* Goode and Bean
2 *Ichthyococcus ovatus* Cocco
1 *Argyropelecus hemigymnus* Cocco
8 *Sternoptyx diaphana* Hermann

conditions have been considered for this comparison, leaving a number of less productive trawlings out of consideration.

1 *Echiostoma ctenobarba ramifer* n. subsp. . . . . See page 17.
1 *Photostomias guernei* Collet
2 *Malacosteus niger* Ayres
1 *Chauliodus sloanei* Schneider
3 *Chauliodus danae* Regan and Trewavas

INIOMI

MYCTOPHOIDEA

1 *Myctophum reinhardti* Lütken
1 *Myctophum laternatum* Garman
8 *Lampanyctus warmingi* Lütken
8 *Lampanyctus subpectoralis* Parr
1 *Lampanyctus photothorax* Parr
2 *Lampanyctus tåningi* Parr
2 *Lampanyctus lineatus* Tåning
4 *Lampanyctus cuprarius* Tåning
2 *Lampanyctus* spp.
1 *Diaphus dumerili* Bleeker
1 *Diaphus* sp.
2 *Omosudis lowei* Günther
1 *Bathypterois nigrescens* n. sp. . . . . See page 19.
1 *Cetomimus kerdops* n. sp. . . . . See page 25.

GIGANTUROIDEA

1 *Gigantura vorax* Regan

LYOMERI

4 *Eurypharynx pelecanoides* Vaillant

APODES

1 *Derichthys serpentinus* Gill . . . . . See page 32.
1 *Serrivomer sector longidentatus* Roule and Bertin
1 *Labichthys carinatus* Gill and Ryder

ANACANTHINI

3 *Laemonema barbatula* Goode and Bean?

BERYCOMORPHI

1 *Gibberichthys pumilus* Parr . . . . . See page 36.
5 *Melamphaes microps* Günther
16 *Melamphaes opisthopterus* Parr
1 *Melamphaes mizolepis* Günther

PERCOMORPHI

PERCOIDEA

1 *Dysalotus alcocki* McGilchrist

SCOMBROIDEA

2 *Decapterus macarellus* Cuvier and Valenciennes

OPHIDIOIDEA

1 *Brotulotaenia crassa* n. sp. . . . . See page 36.

PEDICULATI

CERATIOIDEA

6 *Melanocetus murrayi* Günther
3 *Lophodolus acanthognathus* Regan
1 *Trematorhynchus phyllodon* n. sp. . . . . See page 40.
1 *Linophryne arborifera eupogon* Regan and Trewavas . . . . . See page 46.
1 *Linophryne coronata diphlegma* n. subsp. . . . . See page 54.
1 *Amacrodon binghami* Parr 1927
2 *Borophryne masculina* n. sp. . . . . See page 56.

LIST OF NEW SUBFAMILY, GENERA, SPECIES, AND SUBSPECIES DESCRIBED IN THIS REPORT

Echiostoma calliobarba n. sp.
Echiostoma ctenobarba ramifer n. subsp.
Bathypterois nigrescens n. sp.
Ditropichthyinae n. subfam. (Cetomimidae)
Ditropichthys n. gen.
Cetomimus kerdops n. sp.
Gyrinomimus n. gen.
Gyrinomimus myersi n. sp.
Brotulotaenia crassa n. sp.
Trematorhynchus phyllodon n. sp.
Linophryne coronata diphlegma n. subsp.
Borophryne masculina n. sp.

LIST OF OTHER SPECIES AND SYSTEMATIC GROUPS, OR OTHER SUBJECTS SPECIALLY DISCUSSED IN THIS REPORT

Bonapartia pedaliota and genus *Bonapartia* ("*Zaphotias*"); discussion of synonymies.

GENUS *Cyclothone;* discussion of western Atlantic representatives with figures of *C. microdon* and *C. pallida.*

GENUS *Echiostoma;* key to species, *E. ctenobarba* redescribed with new species and subspecies.

SUBGENUS *Hemipterois* (genus *Bathypterois*); key to species.

Family CETOMIMIDAE; complete revision, new genera; genera *Cetomimus* and *Cetostomus* redefined; figures.

Derichthys iselini Borodin = *D. serpentinus* Gill; figures and description of head.

Genus *Gibberichthys* and *G. pumilus* Parr; large specimen described and figured; with addenda to generic definition.

Genus *Trematorhynchus;* remarks on classification.

Genus *Linophryne;* synoptic re-arrangement of species (key).

Linophryne arborifera eupogon; detailed description and figure of barbel in type; comparison with new specimen and with *L. arborifera arborifera.*

Linophryne coronata coronata Parr; redescribed and figured.

Linophryne coronata longibarbata Borodin; described and figured.

Borophryne masculina; a new male species of Ceratioidea referred directly to its female genus.

SYSTEMATIC ANNOTATIONS

Genus **BONAPARTIA** Goode and Bean

Bonapartia Goode and Bean: Oceanic Ichthyology, 1895, p. 102; Norman: Discovery Rept. Vol. II, 1930, p. 280.

Zaphotias Goode and Bean in: Jordan and Evermann, Fishes of North and Middle America, Vol. III, 1898, p. 2826.

As shown by Norman (loc. cit.) the name *Bonapartia* (Pisces) Goode and Bean antedates *Bonapartia* (Aves) Büttikofer, and is therefore the proper designation for the genus here considered, contrary to customary usage.

Bonapartia pedaliota Goode and Bean

Bonapartia pedaliota Goode and Bean 1895: Oceanic Ichthyology, p. 102, fig. 120; Jordan and Evermann 1896: Fishes of North and Middle America Vol. I, p. 580; Jespersen and Taaning 1919: Vidensk. Medd. Dansk Naturhist. Foren. Vol. 70, p. 221, pl. XVII, figs. 7–8; Norman 1930: Discovery Rep. Vol. II, p. 280, fig. 6.

Zaphotias pedaliota Jordan and Evermann 1898: Fishes of North and Middle America, Vol. III, p. 2826.

Zaphotias nudum Borodin 1930: Proc. New England Zool. Club (Boston), Vol. XI, p. 88; 1931: Bull. Mus. Comp. Zool. Vol. 72, No. 3, p. 71, pl. 2, fig. 2.

? *Zaphotias photocephalus* Roule and Angel 1933: Result. Camp. Sci. Monaco Fasc. LXXXVI, p. 25, pl. I, fig. 13 and 13a.

Due largely to the very misleading original illustration of *B. pedaliota*, considerable confusion and uncertainty has arisen in regard to the accurate definition of this species. To help clarifying the matter, the author has personally examined the type of Borodin's *B.* ("*Zaphotias*") *nudum*, and Dr. G. S. Myers of the U. S. National Museum has very kindly undertaken to make a comparison between the type of *B. pediolata* and the current concept of that species as

represented by a tracing of Norman's figure (Discovery Rept. Vol. II, fig. 6, p. 280).

According to Dr. Myers (*in. lit.*), the type is in complete agreement with Norman's figure both in regard to the spacing and arrangement of the photophores, in the presence of three subcaudal organs, in the form of maxillary and head, and in the forking of the caudal (subtruncate in Goode and Bean's figure). Dr. Myers also makes the observation that "there is no indication of the upper caudal photophore figured by Goode and Bean, even though the fish is uninjured in that region." It is thus evident that the current concept of the species held by such authors as Jespersen and Taaning, Norman, *et al.*, is entirely correct in its interpretation of the form described by Goode and Bean.

The distinction of *B.* ("*Zaphotias*") *nudum* (Borodin) as a separate species is chiefly based upon the proclaimed nakedness of the type. The author has been quite unable to verify this claim, finding, on the contrary, that although the scales have all become lost and the delicate skin is rather shrunken in alcohol, the remnants of the large scale pockets are still very evident under a microscope. There is thus at the present time no reason for accepting *B. nudum* as a valid and distinct species, but it might be pointed out that Borodin's type specimen has only two photophorees in the caudal group and only fourteen on each side in advance of the ventral fins in accordance with what has been described by Jespersen and Taaning as typical of *B. pedaliota* and what is also found in the present specimen. In the type of *B. pedaliota* and in the specimen recorded by Norman (loc. cit.) the caudal group has 3 photophores and Norman gives a count of 16 photophores between gill openings and ventral fins. In the author's opinion this is probably only a matter of individual variation, but if subsequent investigations should give taxonomic significance to the presence of two or of three photophores in the caudal group, Borodin's name would be available for the form in which only 2 organs are found in this position, Goode and Bean's designation referring to the other.

B. ("*Zaphotias*") *photocephalus* (Roule and Angel) is also described as naked and special emphasis is laid upon the presence of 3 photophores on the head, one suborbital, one "opercular" (on the upper part of the gill cover), and one at the corner of the preopercle. These 3 photophores, however, are also present in the types of *B. pedaliota* Goode and Bean (Myers *in lit.*) and of *B.* (*Zaphotias*) *nudum* (Borodin) [Borodin's statement to the contrary (Borodin 1930, *loc. cit.* p. 89) notwithstanding], and in the specimen on hand. They are also all clearly shown in Jespersen and Taaning's figure 7 (*loc. cit.*) and the suborbital and opercular ones are figured by Norman (*loc. cit.*, fig. 6). The photophores on the head are thus not distinctive of *B. photocephalus*, and in view of the highly deciduous character of the scales and the ease with which even the scale pockets may become abrased from the extremely delicate skin, the author feels fairly convinced that *B. photocephalus* should also be listed among the synonyms of *B. pedaliota*.

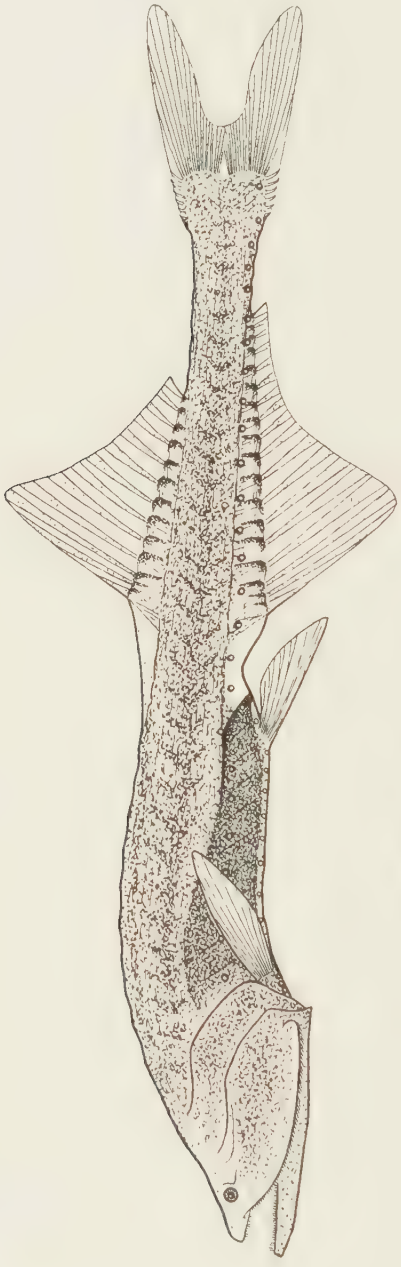

Figure 2. *Cyclothone pallida* Brauer. Drawn by author.

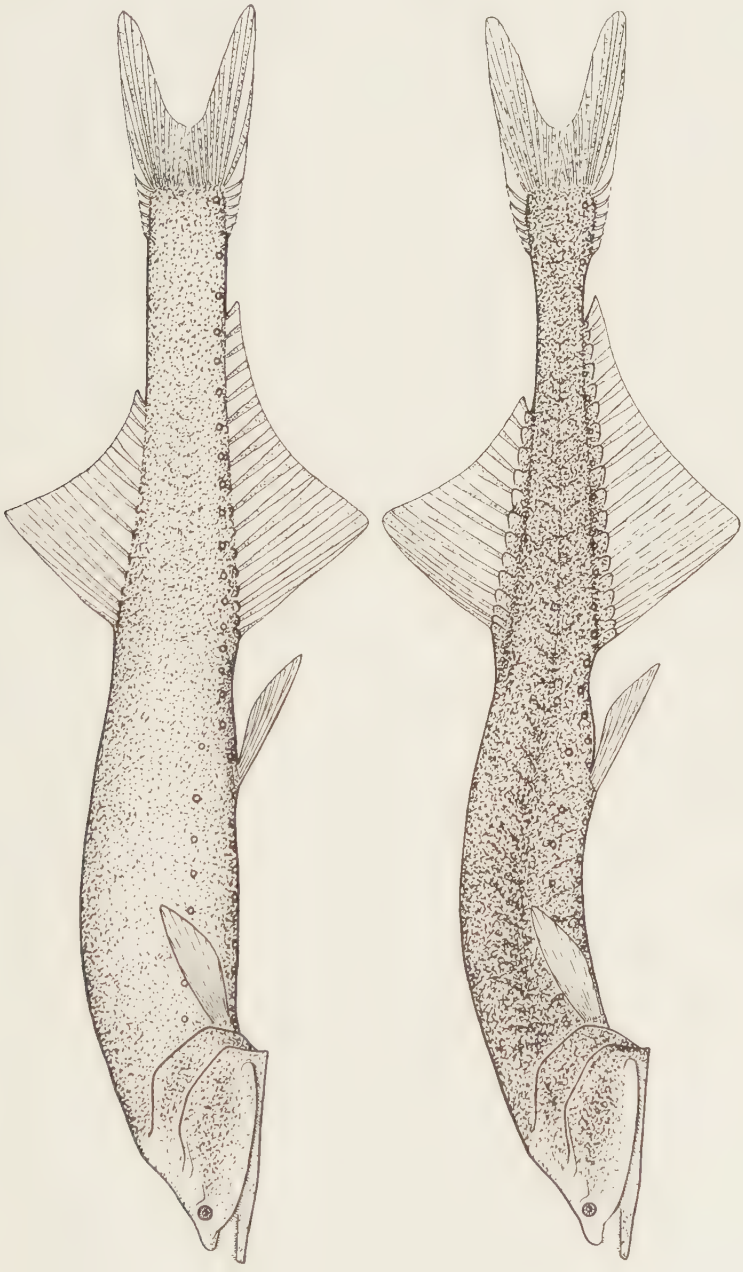

Figure 3. *Cyclothone microdon* Günther. Well nourished (fat) specimen above, emaciated specimen below. Drawn by author.

Genus **CYCLOTHONE** Goode and Bean

Through examination of a great number of samples from the eastern central Atlantic, the author has come to the conclusion that the *Cyclothone* populations of this area consist of 3 species only, the pale *C. braueri*, the brown *C. pallida*, and the brown to black *C. microdon*. Of these *C. braueri* is already so well established and so easily distinguished from the other two that no further comments are required. In formalin preservation and in the fresh material, *C. pallida* is also so conspicuously different from *C. microdon* that the two can be easily separated even by the most casual inspection of a sample. The most striking features of *C. pallida* as compared with *C. microdon* are contributed by the completely pigment-free and transparent ventral area of the skin between its ventral and its anal fin, in the anterior portion of which the black peritoneum lining the tapering end of the abdominal cavity is visible; by the development of internal pigmentation at each pterygiophore in its dorsal and anal fins in alternation with transparent areas between pterygiophores; and to a lesser extent by its generally paler and more transparent skin, with the peritoneum showing through quite clearly in the entire abdominal region and a transparent triangular area generally noticeable in front of the dorsal fin where no opaque tissues are present. In the accompanying figure 2 the author has attempted to show these features as they appear in formalin specimens.[3] Although *C. pallida* has been illustrated twice before (Brauer 1906, Wiss. Ergeb. "Valdivia," Vol. XV, Tiefsee-Fische, pl. VI, fig. 2; Zugmayer 1911, Res. Camp. Sci. Monaco, Fasc. XXXV, pl. II, fig. 2), neither of the previous illustrations give any clear impression of the appearance of the preserved material with which most investigators have to work. In the author's opinion the differences between *C. pallida* and *C. microdon* are such that there is no reason for treating one as a subspecies of the other.

In regard to the material here recorded as *C. microdon*, the author was at first in considerable doubt as to whether it might not include more than one species, the uncertainty being caused by the extremely wide range of variations in general appearance observed among the specimens. It has not been found possible, however, to correlate these variations in appearance with any other feature than the absence or presence and degree of development of the subdermal adipose tissues which appear to be a characteristic of well-nourished specimens of *C. microdon*, but has not been observed at all (macroscopically) in *C. pallida*. This adipose tissue develops particularly along the bases of the dorsal and anal fins and in the median above and below the caudal peduncle, thereby altering the outlines of the peduncle in a most conspicuous manner and completely effacing the sculptural character of the entire tail as shown in the emaciated

[3] In specimens preserved in alcohol, especially if any shrinkage has occurred, the distinctive characters of *C. pallida* are somewhat less striking to the eye, but are usually easily revealed by closer inspection.

specimens (see figure 3). A great development of adipose tissues is also found on the flanks and back of the trunk and also, in a lesser degree, along the sides of the tail; the entire result being a smooth, well-rounded specimen of an oval cross-section as compared with the almost ribbon-like bodies of the emaciated specimens in which all the details of the muscular anatomy are evident on the surface. In formalin specimens the adipose tissues appear as a dense agglomeration of rather large oil globules, wherever the skin is torn to make it visible. In alcohol material the oil has usually been completely extracted and the adipose tissue is shrunken and collapsed. Alcohol material will therefore not show the same range of variation in general shape as formalin specimens or fresh samples, but it seems indicated that an emaciation of the musculature in life occurs parallel with the exhaustion of the adipose tissues so that a specimen which was fat in life will seem relatively corpulent also after the oil has been artificially extracted in alcohol preservation, although it will always lose its smoothness of surface. It also seems evident that emaciation in life is often continued long beyond the complete exhaustion of all adipose tissues so that varying degrees of emaciation is also to be observed among specimens in which no oil at all is left. Since counts and measurements have failed to show any features distinguishing the emaciated from the fat specimens, the author feels confident in referring them all to the same species, with the suggestion that the ability to develop the above described adipose tissues may perhaps in itself be considered a specific character of *C. microdon* although it can only be used for the identification of the specimens in which it has found a morphological expression, i. e. in the well nourished individuals.

Genus **ECHIOSTOMA** Lowe 1843

In their revision of the genus *Echiostoma* Regan and Trewavas (1930, Danish "Dana" Exp. 1920–22. Oceanogr. Rep. No. 6 p. 116) recognize 4 separate species, basing their differentiation upon variations in the bulbs and appendages of the barbel and in the size of the postocular luminous organ. In addition to the specimen caught in the haul here reported upon, two other large representatives of the genus *Echiostoma* are now available in the Bingham Oceanographic Collection, one being the type of *E. ctenobarba* Parr, the other the type of a new species collected by the Atlantis at station 1030.[4] Since each of the three specimens shows considerable differences from the others in the structure and appendages of their barbels, and since the better facilities now available to the author also make a more detailed description and illustration of the barbel of *E. ctenobarba* possible at this time, a brief account will be given in this connection of each of the 3 forms represented by these three specimens. The clearest impression of the mutual relationships of these various species and subspecies may perhaps be obtained from the following key.

[4] The collection also contains three small specimens of *E. tanneri* Gill, which need not be considered in this connection.

I. Barbel with two white bulbs, a longer proximal and a shorter distal one at the end of its main stem, both wider than the stem and sharply differentiated also externally. Distal bulb with terminal filaments.

E. tanneri Gill 1883

II. Barbel with not more than one externally differentiated main bulb, or without. Not as above. With or without additional internal bulb or bulbs.

A. Barbel with simple terminal appendage.

1. "Barbel with bulb not thicker than stem, bearing a simple flat terminal appendage; filaments on stem rather short. Postocular luminous organ 1/5 length of head (in a specimen of 285 mm.)."[5]

E. barbatum Lowe 1843

2. "Barbel with distinct bulb and simple slender terminal appendage; filaments on stem long. Postocular organ 1/4 length of head (in a specimen of 215 mm.)."[5]

E. guentheri Regan and Trewavas 1930

B. Barbel with branched terminal filament.

1. Lateral filaments on stem of barbel small and delicate, each with a minute globular white bulb at its distal end. End of stem marked by a round internal, white body (luminous bulb) beyond which the barbel is continued as a thick, tapering terminal filament which bifurcates twice to form 4 equivalent branches with minor branches distally and a pair of small lateral filaments at the first bifurcation. Length of postocular luminous organ 5 in length of head. (In specimen 290 mm. long).

E. calliobarba n. sp.

2. Lateral filaments on stem of barbel coarser, without terminal bulbs. Length of postocular luminous organ $3\frac{1}{2}$ to $3\frac{2}{5}$ in length of head. (In specimens 275–297 mm. long).

E. ctenobarba Parr 1927

a. Lateral filaments on the main stem of the barbel relatively short and blunt, their series occupying only about 2/5 of the distance from the base of the last (distal) filament to the base of the barbel. Main stem of barbel without visible internal bulb at the distal end.

E. ctenobarba ctenobarba Parr

b. Lateral filaments on the main stem of the barbel long and pointed, their series occupying nearly 1/2 of the distance from the base of the last filament to the base of the barbel. Main stem of barbel with a conspicuous, round, whitish body (luminous bulb ?) internally on the ventral (anterior) side near its distal end..... *E. ctenobarba ramifera* n. subsp.

[5] Regan and Trewavas 1930, *loc. cit.* p. 116.

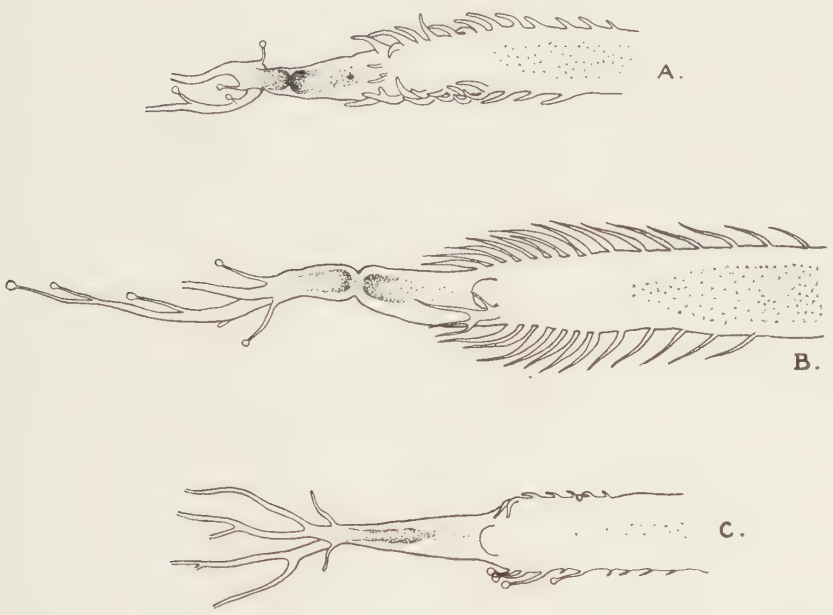

Figure 4. Barbels of *Echiostoma:* A. *Echiostoma ctenobarba ctenobarba* Parr. B. *Echiostoma ctenobarba ramifera* n. subsp. C. *Echiostoma calliobarba* n. sp. Drawings by author.

Echiostoma calliobarba n. sp.

Length without caudal fin 290 mm. Proportions in percent of length without caudal fin: Length of head 15.5. Snout 3.8. Interorbital width 5.52. Diameter of eye 2.20. Lower jaw 13.3. Length of postocular luminous organ[6] 3.03. Greatest depth of body 12.9. Snout to ventrals 57. Origin of dorsal to base of middle caudal rays 17.5. Origin of anal to middle caudal rays 17.0. End of dorsal fin base to middle caudal rays 8.0. End of anal fin base to middle caudal rays 5.5. Length of pectoral filament 40.3. Length of lower pectoral rays 7.9. Length of ventrals 16.7. Distance from base of barbel to distal end of its internal bulb 5.9. Distance occupied by lateral series of filaments on barbel 1.7.

Lateral series of photophores: O–V 25. V–A 17. A–C 11. Ventral series:

I–P 8 + 2. P–V 26. V–A 17.

D 12½. A 16½. V 8. P 1 + 3.

[6] See footnote 9 page 17.

Lateral filaments on the stem of the barbel thin and delicate, each[7] ending in a globular, whitish bulb. About 9 filaments on each side, their series occupying less than 1/3 of the distance from the base of the distal filament to the base of the barbel. A round, whitish, ventrally-situated, internal body (luminous bulb ?), conspicuous from the exterior, marks the end of the main stem of the barbel, which is continued distally by a terminal appendage, the darkly pigmented internal core of which does not appear to have any definite constriction as in *E. ctenobarba* at any point. The terminal appendage bifurcates once, each branch branching again at least once, so that at least 4 main branches are formed, apart from possibly bulb-bearing filaments arising from various parts. There are a pair of opposed filaments at the point of bifurcation of the terminal appendage similar to the filaments found at its first branching point in *E. ctenobarba*. The filaments in *E. calliobarba* are incomplete, however, and it is impossible to say whether they have been bulb-bearing or not. Two of the four ultimate branches have one remannt each of what may have been a small secondary branch or merely a bulb-bearing filament. The main branches themselves are all incomplete at the tip.

Entirely similar to the other species of *Echiostoma*, particularly *E. ctenobarba* in its general appearance.

Type No. 3201 of the Bingham Oceanographic Collection from Atlantis Station 1030; 37° 47′ N. 31° 41′ W., August 4–5, 1931; 15 foot circular net, 610–930 meters vertical depth.

Echiostoma ctenobarba ctenobarba Parr 1927

Echiostoma ctenobarba Parr 1927, Bull. Bingham Oceanogr. Coll. Vol. III, Art. 3, p. 55, figs. 32 and 33; Regan and Trewavas 1930, Danish "Dana" Exp. 1920–22, Oceanogr. Rep. No. 6, p. 116, fig. 112c.

The introduction of a new subspecies of the same species and the better facilities for a detailed examination of a specimen of this size now available to the author makes a redescription of the type specimen seem desirable at this point.

Length without caudal fin 275 mm. Proportions in per cent of length without caudal fin: Length of head 15.6. Snout 3.8. Interorbital width 5.20. Diameter of eye 2.40. Lower jaw 12.2. Length of postocular luminous organ[8] 4.60. Greatest depth of body 15.6. Snout to ventrals 58.7. Origin of dorsal to base of middle caudal rays 17.8. Origin of anal to middle caudal rays 16.5. End of dorsal fin base to base of middle caudal rays 7.6. End of anal fin base to middle caudal rays 5.3. Length of pectoral filament > 35.0. Length of lower pectoral rays 6.6. Length of ventrals 14.5. Distance from base of barbel to base of its ultimate lateral filament 5.1. Distance occupied by lateral series of filaments on barbel 2.2.

[7] i. e., all of the filaments which have been preserved intact in the type specimen.
[8] See footnote 9.

Lateral series of photophores: O–V 25. V–A 16. A–C 12. Ventral series: I–P 8 + 2. P–V 26. V–A 17, only the last organ above the base of anal fin.

D 12½. A 15½. V 8. P 1 + 3.

Lateral filaments on stem of barbel relatively short and blunt, finger-like, without terminal bulbs, their series occupying only about 2/5 of the distance from the base of the distal filament to the base of the barbel. The lateral filaments are arranged in a regular, single series on one side, while the series on the other side is more irregular with the insertions of some of the filaments diverging from the straight line through the rest of the series. The penultimate filament on the side of the irregular series has a stubby branch, and there are three minute, simple filaments inserted in the middle of the ventral (anterior) surface of the barbel between its ultimate lateral filaments. About 13–15 filaments on each side of the barbel. There are no signs of an internal bulb visible from the exterior at the end of the main stem of the barbel.

The terminal appendage continuing the barbel beyond the lateral series of filaments has a darkly pigmented internal core which shows a sharp constriction at a distance in advance of the first branch but a less well marked separation of the parts than in *E. ctenobarba ramifer* (compare fig. 4A and B). The constriction hardly affects the exterior of the terminal appendage at all. The terminal appendage branches once, both branches being partly mutilated in the type, but apparently similar to those in *E. ctenobarba ramifer*, with a pair of opposed, bulb-bearing filaments at their branching point and a couple or more of similar bulb-bearing filaments on one of the branches, at least.

Echiostoma ctenobarba ramifer n. subsp.

Length without caudal fin 297 mm. Proportions in per cent of length without caudal fin: Length of head 14.3. Snout 3.4. Interorbital width 5.0. Diameter of eye 2.25. Lower jaw 12.0. Length of postocular luminous organ[9] 4.3. Greatest depth of body 12.8. Snout to ventrals 57.0. Origin of dorsal to base of middle caudal rays 16.5. Origin of anal to middle caudal rays 16.5. End of dorsal fin base to middle caudal rays 7.5. End of anal fin base to middle caudal rays 4.9. Length of pectoral filament 42.5. Length of lower pectoral rays 6.3. Length of ventrals 12.0. Distance from base of barbel to distal end of its internal bulb 6.4. Distance occupied by lateral series of filaments on barbel 3.0.

Lateral series of photophores: O–V 26. V–A 16. A–C 12. Ventral series: I–P 8 + 2. P–V 27. V–A 17, only the last organ above the base of anal fin.

D 13. A 16½. V 8. P 1 + 3.

Lateral filaments on the stem of the barbel relatively long and pointed, somewhat compressed, particularly the more distal ones; without terminal bulbs; in a single series on each side occupying nearly half of the distance from the base

[9] Measured as the length of the transparent area of the skin covering the luminous parts.

of the distal filament to the base of the barbel. About 13–15 filaments on each side. A round, whitish, very conspicuous internal body (luminous bulb ?) situated ventrally between the last lateral filaments marks the end of the stem of the barbel as here understood. The last lateral filament on one side encroaches upon the ventral (anterior) surface of the barbel beside the internal bulb and carries two branches, the distal one of which is again bifurcated, so that four filaments in all, including the terminal one, arise from the stem of this appendage (see figure 4B).

The terminal appendage continuing the barbel beyond the internal bulb has an inner darkly pigmented core which is sharply subdivided by a constriction about halfway between the internal bulb and the base of the first branch. This constriction is well marked also externally. Beyond the constriction the terminal appendage branches once, but due to mutilation of one of them it is impossible to tell whether the branches have been equivalent or not. The unmutilated branch carries three thin lateral filaments, one incomplete, and the other two ending in a small, globular, whitish bulb each. The branch itself terminates in a similar but slightly larger bulb and there is an opposed pair of similar bulb-bearing filaments at the branching point of the terminal appendage.

General appearances in the other species of *Echiostoma*.

Type of the subspecies No. 3202 of the Bingham Oceanographic Collection Atlantis Station 1478.

Genus **BATHYPTEROIS** Günther

Subgenus HEMIPTEROIS Regan

Hemipterois Regan 1911: Ann. Mag. Nat. Hist. Ser. 8, Vol. VII, pp. 126 and 127. Parr 1928: Bull. Bingham Oceanogr. Vol. III, Art. 3, p. 25.

Belonepterois Roule 1916: Bull. Inst. Oceanogr. Monaco No. 320, p. 13; 1919: Res. Camp. Sci. Monaco Fasc. LII, p. 35.

As subgenus *Hemipterois* is here considered the species with 6 or more rays in the upper portion of the pectoral fins, without a notch in the ventral outline at the base of the lower caudal rays, and with the outer ventral and lower caudal rays always, and the upper pectoral rays sometimes, strongly produced. As opposed to these forms, the species with only four or less rays in the upper portion of the pectoral fins are arranged in the subgenus *Bathypterois* (see Parr 1928, loc. cit.).

The specimen collected at Atlantis station 1478 adds a third species of subgenus *Hemipterois* to the two previously known. These species may be distinguished as follows:

Key to subgenus *Hemipterois*

1. Longest (upper) pectoral ray rigid and enormously produced reaching to or beyond the end of caudal fin. Only 5 rays in lower portion of pectoral fin.

Dorsal origin a little in advance of the middle of the body, anal origin immediately behind the end of the dorsal fin base.

Bathypterois (*Hemipterois*) *guentheri* Alcock

2. Longest rays in upper portion of pectorals not reaching to the adipose dorsal fin. Origin of anal fin approximately below middle of dorsal fin base.
   A. Dorsal origin a little in advance of the middle of the body.[10] Only 5 rays in the lower portion of pectoral fin. Color light gray.[11]

      Bathypterois (*Hemipterois*) *viridensis* Roule

   B. Dorsal origin immediately behind the middle of body. 6 rays in lower portion of pectoral fin. Color black (see description).

      Bathypterois (*Hemipterois*) *nigrescens* n. sp.

Bathypterois (Hemipterois) nigrescens n. sp.

Length without caudal fin 61 mm. Proportions in per cent of length without caudal fin: Snout to dorsal fin 51.6. Length of head 27.4. Snout to eyes 8.7. Interorbital space 8.0. Maxillary 16.7. Length of dorsal fin base 14.7. Snout to anal fin 59.0. Longest caudal ray 59.0. Longest ventral ray 69.7. Longest pectoral ray (upper portion) 23.0. Snout to ventral fins 43.8. Greatest depth of body (deflated) 18. Least depth of caudal peduncle 8.9.

D. 12. A. 11. V. 8. P. (1–2 + 7) + (6). L1. 50.

In general appearance the type of *B. nigrescens* seems slightly shorter and somewhat chunkier than a specimen of *B. viridensis* of comparable size. This effect is partly due, however, to a liquid inflation between skin and musculature which makes it difficult to determine the natural proportions of *B. nigrescens* in regard to body-width and depth, but it seems indicated that the apparent relative stubbiness of this form is at least in part natural to the species. There are otherwise no features in the general appearance of *B. nigrescens* to distinguish it from the other known species of the same genus.

The upper portions of the pectoral fins each consist of 7 rays, plus two rudimentary rays closely applied to the upper and longest normal ray. The rays in the upper portion of the fin are united by fin membrane and are much more closely set than are the rays in the lower portion. The lower portions of the pectorals each contain 6 about equally spaced free rays, of which the lowest is the longest but unfortunately incomplete on both sides. In *B. viridensis* the interspace between the two portions of the pectorals is greatly increased as compared with the interspaces between the rays in the lower portion itself. In *B. nigrescens* this interspace is divided by the 6th lower ray so that the spacing of the lower portion continues to the first ray in the upper.

The produced ray in each ventral fin splits in two approximately opposite the base of caudal, beyond which they extend for an unknown distance, their

[10] Snout to dorsal fin 47.7–48.5 per cent of length without caudal.

[11] Observed by the author on the newly caught specimen.

ultimate portions apparently being lost. Both of the two produced lower caudal rays also divide distally, but it is questionable whether the division of the produced rays in this specimen may not be accidental in all cases.

The skin has apparently been subject to considerable rubbing in the net, but wherever the pigmentation is preserved, whether on back, sides, abdomen, or tail, it indicates a uniform black coloration, in contrast with the light peppered gray of *B. viridensis*. The caudal fin is also black, the other fins pale.

Type No. 3203 of the Bingham Oceanographic Collection Atlantis station 1478.

FAMILY **CETOMIMIDAE**

From a comparison of the new cetomimid specimen obtained by the "Atlantis" at station 1478 with the other examples of this family already available in the Bingham Oceanographic Collection, it immediately became apparent, on the basis of external appearances alone, that at least two entirely distinct generic types were present in the material. Further examination also soon divulged the existence of a third taxonomic type, represented by the so-called *Cetomimus storeri*, which appears to stand so far apart from the other cetomimid forms that at least a separate subfamily is required for its classification. A revision of the entire family thus became necessary and in this effort the author has been greatly aided by the information concerning the type of *Cetomimus gilli* kindly supplied by Dr. G. S. Myers of the U. S. National Museum on the basis of a comparison with the type of *Gyrinomimus myersi* n. gen. et n. sp.

With the sharpened generic definitions, it also seems appropriate to resurrect the genus *Cetostomus* Zugmayer which the writer has previously synonymized with *Cetomimus* Goode and Bean, with which it is closely related.

Key to the Cetomimidae[12]

I. Gills 4, a moderate slit behind the fourth. Gill rakers present.

Subfamily Ditropichthyinae

A pair of thin dermal folds along the entire ventral edge of the abdomen, continued posteriorly as a free, curtain-like, skin fold on each side of the

[12] The genus *Pelecinomimus* Gilchrist (Rep. Fish. Mar. Biol. Surv. Union of S. Africa, Vol. II, p. 56) is described as having "very long scales," but these scales are not shown in the figure of the type-species, which, on the contrary, seems to suggest a typical naked cetomimid form, as already pointed out by Barnard (Ann. S. African Mus. Vol. XXI, Pt. 1, 1925, p. 252). If scales are actually present, the genus would occupy an entirely separate position, if not, it might very likely prove to be only a synonym for *Cetomimus* Goode and Bean. As it stands at present, there is too great uncertainty attached to its definition to make it possible to include it in the above key or to deal further with it in the text.

anal fin base. Teeth small, villiform, in irregular bands in jaws, vomer, and palatines. Gill rakers thick, club-shaped; present on all arches.

Genus *Ditropichthys* n. gen.

Ditropichthys storeri Goode and Bean

II. Gills 3, a small slit behind the third. Gill rakers absent.

Subfamily Cetomiminae

A. Teeth minute, short and broad ("granular") in irregular bands in the jaws, on vomer, palatines, pterygoids, gill arches and tongue, without forming a specially differentiated oval shield on the latter. Head not tadpole-like.

1. Dorsal and anal fins moderate (D 20 or less, A 17 or less). Trunk relatively robust.

Genus *Cetomimus* Goode and Bean

a. Head not much compressed, less than 3 times in the length without caudal. Distance from origin of dorsal fin to end of hypural about 4, greatest external width between rami of lower jaw about 7 in length without caudal.

C. gilli Goode and Bean

b. Head compressed, more than 3 times in the length without caudal. Distance from origin of dorsal fin to end of hypural about 3 1/4. greatest external width between rami of lower jaw nearly 12 in length without caudal.

C. kerdops n. sp. ad. int.

2. Dorsal and anal fins long (D about 30, A about 27). Trunk very attenuate. Tail at D and A high and compressed. Head compressed.

Genus *Cetostomus* Zugmayer

Cetostomus regani Zugmayer

B. Teeth very small, but slender and elongate, arranged in a number (3–5) of very close-set and extremely precise series in the jaws. Similar teeth on vomer, palatines, and pterygoids. An oval shield of concentrically-arranged curved teeth on the tongue. Slightly elongate teeth in gill arches. Head broad, depressed, tadpole-like.

Genus *Gyrinomimus* n. gen.

Gyrinomimus myersi n. sp.

DITROPICHTHYS New Genus

Gills 4, a small opening behind the fourth. Thick, club-shaped, denticulate gill rakers present on all of the four free arches. Eyes small but not rudimentary. Head and body compressed, naked. Lateral line very wide, prominent, semi-tubular, with few, enormous pores. Pectorals low. Dorsal and anal fins high,

with about 20 or less rays of fairly uniform length giving the fins a rectangular rather than triangular outline. A deep, free skin fold with a lobate edge (see figure) extends along each side of the base of anal fin, while a pair of simple-edged dermal folds occupy the ventral distance from anal opening to shoulder girdle. Mouth very large. Teeth small, in villiform bands on jaws, vomer, palatines, pterygoids and in a short patch on the tongue. Lateral line pores on trunk and tail few (about 12), large and simple, without flaps.

Genotype: *Ditropichthys storeri* (Goode and Bean).

At the end of their description of *Cetomimus storeri*, Goode and Bean (1895, Oceanic Ichthyology, p. 69) make the statement that the type of the species was no longer available at the time when they made their studies of the cetomimid fishes in preparation of their report, their account of *C. storeri* being based solely upon a drawing previously prepared under the supervision of Dr. Bean. From this it is evident that the generic definition of *Cetomimus*, except in so far as the purely external features are concerned, must have been based upon *C. gilli* only, but this implication of Goode and Bean's statement has unfortunately been overlooked in the two subsequent new records and redescriptions of *C. storeri* published by Brauer (Die Tiefsee Fische. I Syst. Teil. Wiss. Erg. Valdivia Vol. 15, 1906, p. 252, pl. X, fig. 5), and by the present writer (Parr, Bull. Bingham Oceanogr. Coll. Vol. III, Art. 3, 1928, p. 177, fig. 43), and it has thus been left for the report in hand to introduce the discovery that "*Cetomimus*" *storeri*, far from being a congener of *Cetomimus gilli*, actually occupies a very isolated position in the family Cetomimidae in a separate genus and subfamily.

The above analysis of this genus and the following account of its only known species is based upon specimen No. 2134 of the Bingham Oceanographic Collection.

Ditropichthys storeri (Goode and Bean)

Cetomimus storeri Goode and Bean: Proc. U. S. Nat. Mus., Vol. 17, (1894) 1895, p. 453, pl. 17, fig. 3; Oceanic Ichthyology, 1896, p. 69, fig. 79; Jordan and Evermann: Fishes of North and Middle America, Vol. I, 1896, p. 550; Brauer: Die Tiefsee Fische I Syst. Teil. Wiss. Erg. Valdivia, Vol. 15, 1906, p. 252. pl. X, fig. 5; Parr: Bull. Bingham Oceanogr. Coll. Vol. III, Art. 3, 1928, p. 177, fig. 43).

Length without caudal fin (to end of hypural) 27 mm. Proportions in per cent of this measurement: Length of head 38.9. Length of snout 14.8. Diameter of eye 3.0. Length of maxillary 24.1. Length of lower jaw 25.9. Depth of head 29.6. Greatest width of skull 16.7. Interocular width 11.9. Height of dorsal dermal crest 6.6. Depth of supra-anal skin fold 2.2. Snout to dorsal fin 65.5. Base of dorsal fin 23.7. Base of anal fin 17.4. Distance from base of anal fin to end of hypural 15.2. Depth of caudal peduncle 6.0.

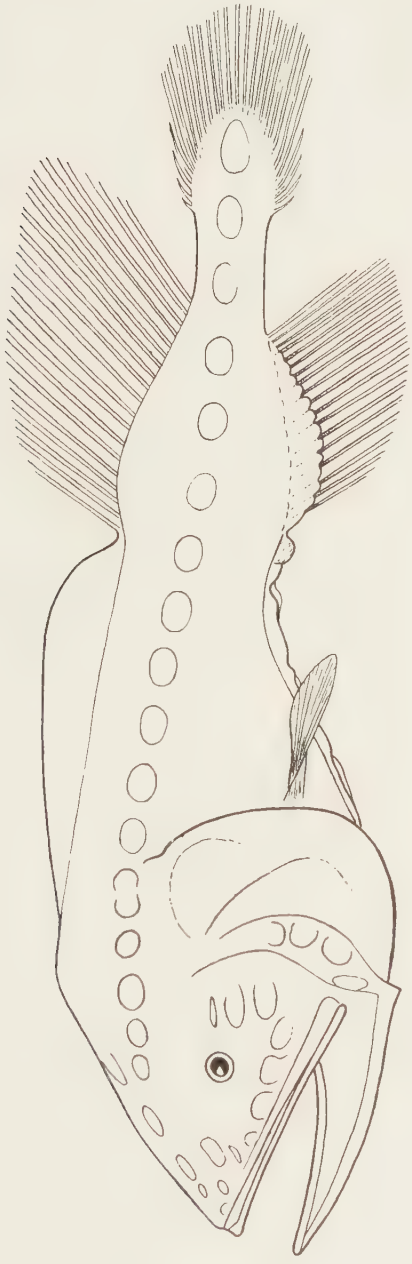

Figure 5. *Ditropichthys storeri* (Goode and Bean). Drawn by Y. H. Olsen.

D 19–20. A 16–18.[13] Lateral line with 12 enormous pores between the shoulder and its posterior end on the caudal fin. There are 3 gill rakers in the upper portion, 1 at the angle, and 8 in the lower portion of the first arch. The supra-anal skin folds terminate at the base of the fourth or fifth anal ray counted from behind, their edges showing 13 lobate subdivisions each.

Known from the Atlantic and Indian Oceans (3 specimens).

Genus **CETOMIMUS** Goode and Bean, *sensu stricto*

Cetomimus (partim) Goode and Bean: Proc. U. S. Nat. Mus. Vol. XVII, 1894 (1895), p. 452; Oceanic Ichthyology, 1895, p. 68; Jordan and Evermann, Fishes of North and Middle America, Vol. I, 1896, p. 549; Brauer, Die Tiefsee Fische. I. System. Teil. Wiss. Erg. Valdivia, Vol. XV, 1906, p. 251; Parr, Bull. Bingham Oceanogr. Col. Vol. III, Art. 3, 1928, p. 173.

Gills 3, a small opening behind the third. Gill rakers absent. Eyes minute. Head not tadpole-like, generally more or less compressed. Trunk and tail compressed. Mouth very large. Lower jaw with a prominent lateral bony protuberance behind the end of the upper jaws. Dorsal and anal fins with less than 20 rays each, the rays being graduated in length so as to give the fins a pointed, oblique, triangular outline.[14] Caudal moderate. Pectorals short, low, but lateral. Teeth short, stubby, "granular"; in a band of 3–5 alternating rows in each jaw. Similar teeth in a short patch on vomer, in bands on palatines and pterygoids and on the gill arches. Lingual patch of teeth long, narrow, and irregular. Lateral line pores on head and body simple, about 20[15] or more between the top of the gill slit and the end of the tail.

Genotype *C. gilli* Goode and Bean.

Cetomimus gilli Goode and Bean

Cetomimus gilli Goode and Bean. Proc. U. S. Nat. Mus. Vol. XVII, 1894 (1895), p. 452, pl. 17, fig. 2; Oceanic Ichthyology, 1895, p. 69, fig. 78; Jordan and Evermann, Fishes of North and Middle America, Vol. I, 1896, p. 549.

? *Cetomimus gilli* Brauer: Die Tiefsee-Fische. I. System. Teil. Wiss. Erg. Valdivia Vol. XV, 1906, p. 251.

Nec. *Cetomimus gilli* Parr: Bull. Bingham Oceanogr. Coll. Vol. III, Art. 3, 1928, p. 176.

From the measurements given by Brauer (loc. cit. p. 252). The Valdivia specimen would seem to agree with *C. kerdops* rather than with *C. gilli* in regard to the length of the head (31.3 per cent of body length; 36.4 per cent in the type of *C. gilli*)[16] and the distance from origin of dorsal to base of caudal fin

[13] Previous count erroneously too low.

[14] Described from *C. kerdops*. Probably also applies to *C. gilli*.

[15] 21 pores are vaguely indicated in Goode and Bean's figure of *C. gilli* (Oceanic Ichthyology, fig. 78, pl. xxi) and approximately 25 are counted in *C. kerdops*.

[16] The author is indebted to Mr. George S. Myers of the U. S. National Museum for furnishing these measurements of the type of *C. gilli*.

(snout to origin of dorsal 67.7 per cent of body length, leaving 32.3 per cent as a minimum for the distance from origin of dorsal to base of caudal which measures 25 per cent in the type of *C. gilli*).[17] The interorbital width of Brauer's specimen is, on the other hand, greater (14.6 per cent of body-length) even than that in the type of *C. gilli* (13.4 per cent of length without caudal[18]). It is therefore not unlikely that Brauer has actually been dealing with a third, independent species.

The characters differentiating *C. gilli* from *C. kerdops* are given in the key on page 21.

Cetomimus kerdops n. sp.

Length without caudal fin 47.5 mm. Proportions in per cent of this measurement. Length of head (snout to pectorals) 31.6. Length of maxillary 28.2. Length of lower jaw 31.0. Snout to eye 12.6. Interorbital width (= width of skull) 10.5. Greatest external width between rami of lower jaw 8.4. Snout to top of gill slit 24.9. Width between tops of gill slits 5.9. Length of pectorals 9.5. Snout to origin of dorsal fin 71.1. Base of dorsal fin 17.5. Base of anal fin 17.5. Snout to anal fin 72.2. Longest rays in dorsal and anal fins 15.8. Greatest depth 18.7. Greatest height between bases of dorsal and anal fin rays 12.6. Least depth of caudal peduncle 5.9. Diameter of eye about 2.0.

D. 16. A. 17. P. 19 (approx.). About 25 pores in the lateral line between top of gill slit and end of tail.

A mosaic band of three alternating rows of teeth in each jaw. Dentition on other parts as described in the generic definition.

Dorsal profile of snout slightly concave. Antero-lateral corners of skull (frontals ?) forming conspicuous prominences at the last third of the distance from the snout to the eyes which are situated laterally below the broadest part of the cranium. Mouth strongly oblique, lower jaw slightly prominent at the tip. A short, sturdy, downwardly-directed spine is formed by the angular at the posterior end of the lower jaw. Above this angular spine a spine-like lateral protuberance arises from the side of the lower jaw with a receiving concavity for the end of the upper jaws anteriorly. The body is somewhat arched with head pointing downwards, and its outlines smooth. Dorsal and anal fins with triangular profiles. Uniform black, fins included.

Type specimen No. 3204 of the Bingham Oceanographic Collection. Atlantis station 1478.

Genus **CETOSTOMUS** Zugmayer

Cetostomus Zugmayer, Bull. Inst. Oceanogr. Monaco No. 288, 1914, p. 4.
Cetomimus (partim) Parr, Bull. Bingham Oceanogr. Coll. Vol. III, Art. 3, p. 173. Occas. Pap. Bingham Oceanogr. Coll. No. 2, 1929, p. 23.

[17] Myers, *in lit.*
[18] Cf. footnote on preceding page.

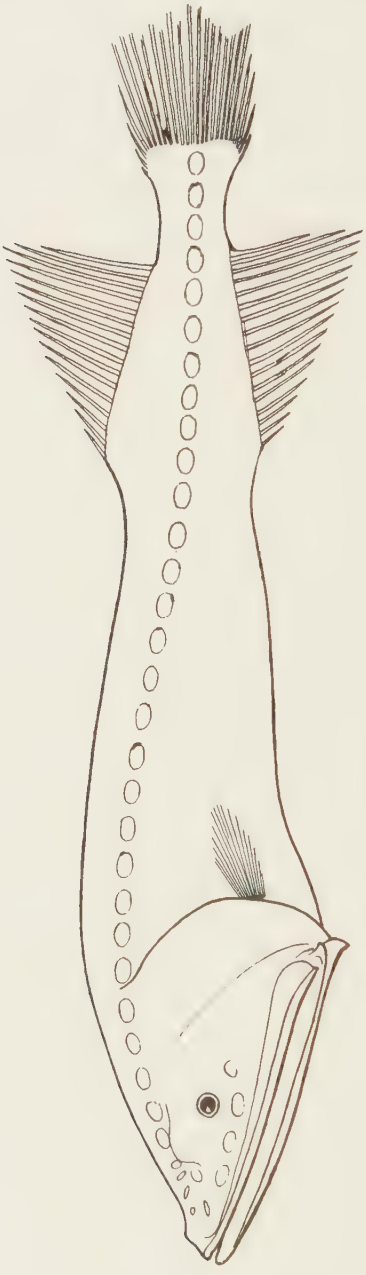

Figure 6. *Cetomimus kerdops* n. sp. Drawn by Y. H. Olsen.

Gills 3, a small opening behind the third. Gill rakers absent. Eyes rudimentary. Head deep, strongly compressed. Trunk very attenuate. Dorsal and anal fin bases elevated, compressed. Caudal peduncle, slender, compressed. Caudal moderate, only slightly emarginate. Pectorals very low. Dorsal and anal fins with numerous rays (D about 30, A about 27) of fairly uniform length, giving to these fins a rectangular rather than triangular outline. Mouth very large. Articular bone of lower jaw without lateral protuberance behind end of maxillary. Teeth minute and stubby, in dense bands on jaws, palatines, pterygoids, and throughout the entire length of the long and very narrow tongue, in a heart-shaped patch on vomer, and in a series of patches on each of the three free gill arches, and on the upper pharyngeals.

Genotype: *Cetostomus regani* Zugmayer.[19]

Cetostomus regani Zugmayer

Cetostomus regani Zugmayer, Bull. Inst. Oceanogr. Monaco No. 288, 1914, p. 4.
Cetomimus regani Parr, Bull. Bingham Oceanogr. Coll. Vol. III, Art. 3, 1928, p. 174, fig. 42; Occas. Pap. Bingham Oceanogr. Coll. No. 2, 1929, pp. 23–27, figs. 9–10.

In conjunction with the revision of the other species of Cetomimidae available to the author, a more complete set of measurements of *C. regani* may be in place. Although the specimen has been stained with alizarin and has been cleared and kept in glycerin since 1928, in which process its length without caudal has shrunk from 141 mm. to 130 mm., it does not appear to have changed significantly in its proportions. This may be seen from a comparison of the following new measurements with the old records of some of the proportions in the fresh (formalin) specimen.

Length without caudal fin 130 mm. (141 mm., *1928*). Proportions in per cent of these measurements: Length of head 30.0 (29.1—*1928*). Greatest width of skull 9.2 (9.2—*1928*). Snout to eye approximately[20] 11 (10.6—*1928*). Length of maxillary 21.7 (20.6—*1928*). Length of lower jaw 23.1 (21.3—*1928*). Snout to end of skull 18.5. Greatest depth of head (supraoccipital to corner of angular) 19.6. (Least depth of trunk anterior to vertical fins 5.0—*1928*). Least depth of caudal peduncle 3.5 (3.5—*1928*). Greatest height between bases of dorsal and anal rays 13.1 (12.8—*1928*). Height of caudal fin base 7.9. Snout to dorsal fin 58 (57—*1928*). Length of pectorals 9.2. Length of caudal 10.8. Longest dorsal ray 11.5. Longest anal ray 10.0. Origin of dorsal fin to end of hypural

[19] The analysis of the genus and species is here based upon specimen No. 2132 of the Bingham Oceanographic Collection. The author is indebted to Dr. Richard of the Monaco Museum for verifying and confirming the identification by comparing the previously published description and figure of the Bingham Collection specimen (Parr, 1928, loc. cit.) with the type in the Monaco collection.

[20] Both eyes partly detached, accurate position therefore somewhat uncertain, which was true also in 1928.

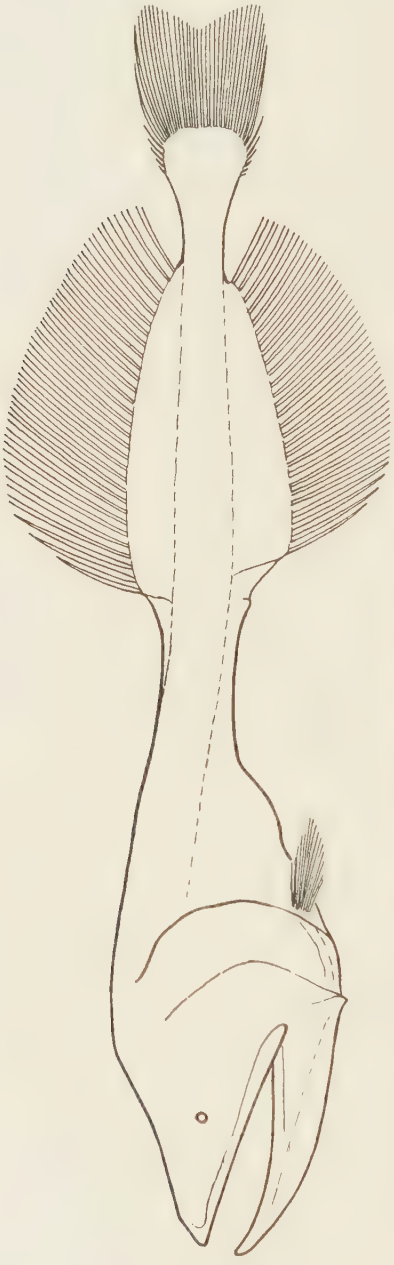

Figure 7. *Cetostomus regani* Zugmayer. Drawn by Y. H. Olsen.

42.3 (43.3—*1928;* base of dorsal 30.5 + caudal peduncle 12.8). Origin of anal fin to end of hypural 39.6. End of dorsal fin base to end of hypural 13.3 (12.8—*1928*). End of anal fin base to end of hypural 14.2.

D. 30. A. 27. P. 18. 51 vertebrae.

Known only from the Atlantic (2 specimens).

GYRINOMIMUS New Genus

Gills 3, a small opening behind the third. Gill rakers absent. Eyes minute. Head broad, depressed, tadpole-like. Snout transverse, mouth widely arched. Tail moderately compressed, caudal peduncle slender. Dorsal and anal fins with less than 20 rays each, the rays increasing conspicuously in size towards the posterior rounded corners of these fins. Caudal moderate, slightly emarginate. Pectorals very low, ventral, inserted anteriorly to the posterior end of the gill cover. Mouth very large. Lower jaw without lateral protuberance. Jaws with small, slender, subequal teeth arranged in a number of very precise, close-set series. Similar teeth on vomer, palatines and pterygoids. An oval shield of concentrically-arranged, curved teeth on the tongue. Maxillary dentition posteriorly entirely confined to the external surface. Slightly elongate small teeth in the gill arches. Lateral line pores on the head large, wide and simple, those of the trunk and tail, about 15 in number, with a pointed flap from their anterior margin increasing in size towards the tail until it completely covers the lateral aspect of the pores on the caudal peduncle. Outer wall of lateral line tube, and flaps, with a series of minute papillate organs externally (see dorsal view, fig. 8). Isolated papillae of a similar nature on the nape, and a semicircular pattern of minute pores above and behind the eyes.

Genotype: *Gyrinomimus myersi* n. sp.

Gyrinomimus myersi n. sp.

Cetomimus gilli (*nec.* Goode and Bean) Parr: Bull. Bingham Oceanogr. Coll. Vol. III, Art. 3, 1928, p. 176.

Length without caudal fin (to end of hypural) 108 mm. Proportions in per cent of this measurement: Length of head 32. Length of maxillary 29.3. Length of lower jaw 27.8. Interocular width 15.7. Interorbital width (skull) 10.9. Snout to eye 12.5. Greatest external width between rami of lower jaw 22.2 in the undisturbed specimen as preserved, minimum possible natural value for this measurement 19.5 per cent. Width between tops of gill slits 14.8. Snout to top of gill slit 24–25. Snout to pectorals 27.8. Length of pectorals 8.5. Snout to origin of dorsal fin 71.7. Origin of dorsal to base of caudal fin 30.6. Base of dorsal fin 17.6. Snout to origin of anal fin 70.5. Base of anal fin 15.7. Greatest depth 25.9. Depth at origin of dorsal fin 16.7. Least depth of caudal peduncle 6.0.

D. 16½. A. 16½. C. 19. P. 19. 15 pores in lateral line from top of gill opening to end.

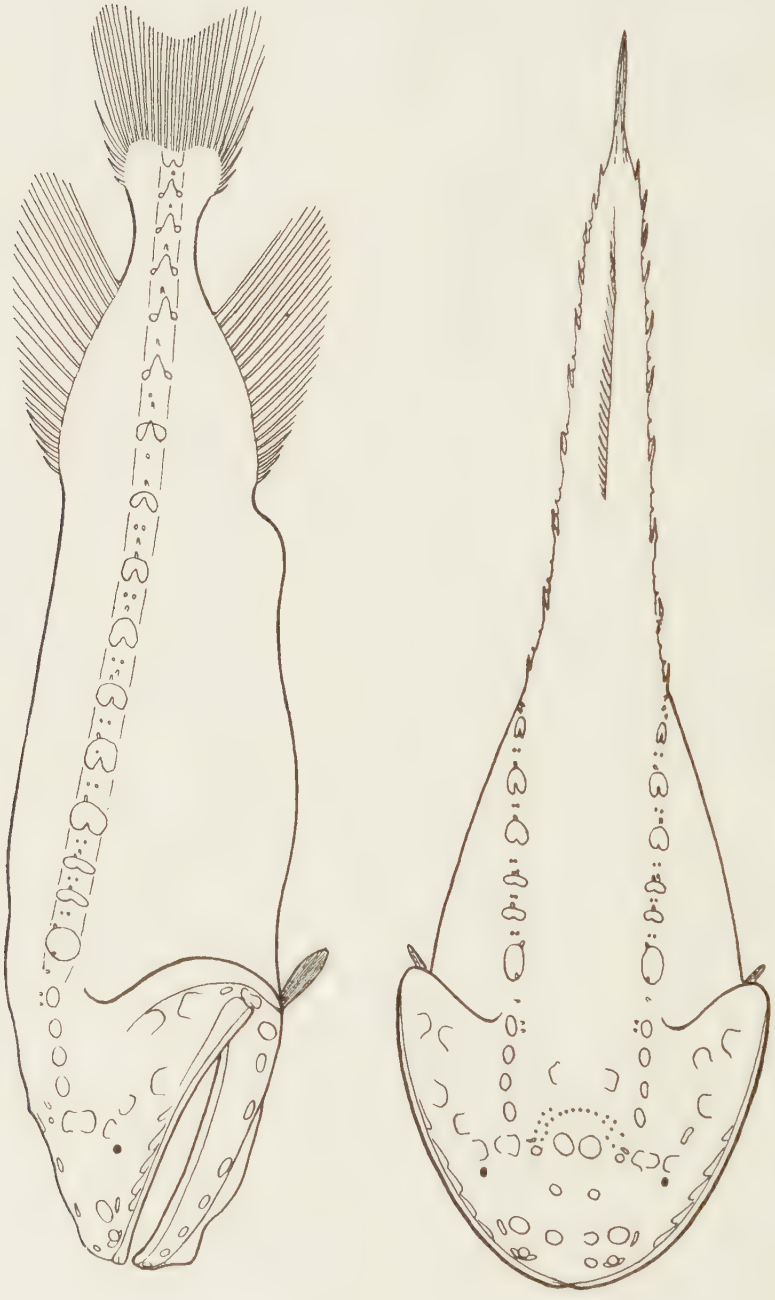

Figure 8. *Gyrinomimus myersi* n. sp. Lateral view above; dorsal view below. Drawn by Y. H. Olsen.

Head broad, depressed, with the appearance of being cavernous under the skin. Mouth enormous, oblique. Jaws slightly arched (artefact ?) in lateral view. Lower jaw without spines or prominences posteriorly. Dentition in the jaws as shown in figure 9. The upper jaw starts with 3 series of teeth (the main series of the upper jaw) anteriorly increasing in size inwards. The inner two

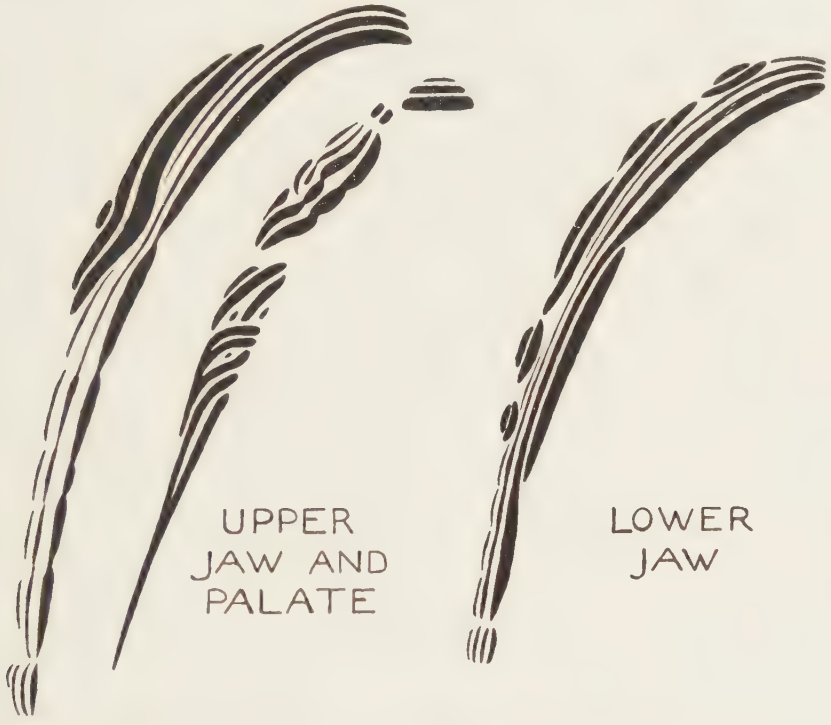

Figure 9. Diagram of upper and lower dentition-pattern in *Gyrinomimus myersi* n. sp. Width of band used to indicate size of teeth in series. Prepared by author.

series are continuous throughout the length of the premaxillary, the third also extending with interruptions to the end of the jaw. A double series of relatively large teeth with a couple of teeth of a third row form an elongate patch external to the three inner series of teeth on the anterior half of the jaw. A fragment of a fourth series is also to be found in the terminal patch of teeth in the upper jaw otherwise formed by semi-separated segments of the three main series of dentition. No separate patch of inner teeth in upper jaw. The dentition of the lower jaw starts with four separate series at the anterior end (the four "main" series of the lower jaw), increasing in size towards the interior of the mouth.

Of these series the inner two are continuous and the third extends discontinuously to the end of the dentary. The fourth (outer) series, however, is soon interrupted by a series of external patches of larger dentition, each patch being made up of a double row of teeth. These external patches of teeth in the lower jaw seem variable both in regard to their number and the length of each. In the lower jaw there is also an extra internal patch of dentition consisting of a single row of relatively large teeth in the middle portion of the jaw. Terminal patch of teeth in lower jaw with four short series.

In the inner two rows of teeth in the upper jaw in particular, less conspicuously elsewhere, there is a tendency for the series to be divided into segments characterized by a decrease in the size of the teeth towards the posterior end of one of these segments followed by a sudden increase at the anterior end of the next. These segments are also roughly indicated in the figure. The arrangement of the teeth in the jaws is not only striking by the absolute perfection of the longitudinal line-up of the series but also by the extreme precision of the transverse alternation of teeth in the main series[21] in which the numbers of teeth do not increase with a decrease in their size, each series on the contrary being in exact correspondence with the others in regard to counts and alternate arrangement at all points. The external patches of dentition in upper and lower jaw and the internal patch in the lower form separate systems in which the numbers and insertions of the teeth do not join the pattern of the corresponding parts of the main series. Vomer with three precise transverse series of teeth forming a triangular group pointed forward; the inner series being the longest and having the largest teeth. Palatine with a system of undulating series of teeth. A similar pattern (see figure 9) is also observed in the anterior broad portion of the pterygoid dentition which is continued backwards as a double series of teeth fusing into one posteriorly. The palatine and pterygoid dentition is somewhat less precise in its pattern than are the series in the jaws and on vomer; but the several series are still very distinctly defined.

Profiles of dorsal and anal fins obliquely oval. Caudal short, slightly emarginate. Uniform brownish-black, fins included (purplish-black in life).

Named for Dr. George S. Myers of the U. S. National Museum.

Type specimen No. 2111 of the Bingham Oceanographic Collection. Pawnee 1927, station 18, March 18, 23° 39′ N. 76° 41′ W., 7000 feet wire (approximately 700 fathoms vertical depth). Only the type known.

Genus **DERICHTHYS** Gill

Derichthys serpentinus Gill

Derichthys serpentinus Gill 1887, American Naturalist, Vol. XVIII, p. 433; Goode and Bean 1895, Oceanic Ichthyology, p. 161, fig. 169.

[21] 3 in upper jaw, 3 + fragment of fourth anteriorly in lower jaw.

Derichthys iselini Borodin 1929, Proc. New England Zool. Club, Vol. X, p. 110; 1931, Bull. Mus. Comp. Zool. Vol. LXXII, No. 3, p. 75, pl. III, fig. 2.

It is unfortunate that the condition of the type specimen of *D. serpentinus* no longer makes possible any comparison with the described characters of *D.*

Figure 10. Head of *Derichthys serpentinus* Gill. Dorsal view above; lateral view below. Arrows indicate true anterior nostril. Y. H. Olsen and author.

iselini, but since, on the other hand, the existing description and figure of *D. serpentinus*, although relatively lacking in details, show no features which are not also common to *D. iselini*, it seems clear that Gill's specific designation should have been considered available for Borodin's specimen. A multiplication of the nomenclature is therefore at present devoid of any meaning whatever

and may only become justified if future investigations should serve to establish the actual existence of more than one species in the North Atlantic, but there is no evidence as yet to suggest this possibility.

The statements as to the conspicuousness or inconspicuousness of the lateral line are obviously highly subjective, dependent upon the comparison one has in mind. The author would personally be inclined to consider the lateral line as being neither conspicuous nor inconspicuous. Distinction for *D. iselini* has also been claimed by Borodin on the basis of the character and arrangement of its nostrils, but a careful probing of the peculiar anterior pair of pores on the snout described by Borodin as anterior nostrils has failed to show any relations to the nasal organs at all, although a tortuous connection might still be possible, *while, on the other hand, one of the lateral pores farther back represents a perfectly normal anterior nostril in accordance with Gill's description.* That the peculiar shape of the dorsal fin may have been overlooked or interpreted as purely accidental and therefore omitted in the original description and figure is not at all surprising when we consider the nature of this character and the fact that only one single, possibly shrunken, alcohol specimen was available at the time. Borodin's statement (1931, *loc. cit.* p. 75) that *D. iselini* should differ from *D. serpentinus* by the shape of its dorsal fin is therefore not warranted by any positive knowledge of the actual shape of this fin in the type of *D. serpentinus* and can be disregarded so long as no evidence is available to show the existence of two separate species differing on this point. There is thus altogether no positive reason at all for maintaining *D. iselini* as distinct from *D. serpentinus.*

The accompanying figure shows in detail the distribution of pores and dermal striations on the head in the specimen here reported upon, with arrows pointing to the true anterior nostril. The pattern of pores and striations shown in this figure agrees in detail with that found in the type specimen of *D. iselini* with which the drawings have been compared.

The dermal striations consist of low but sharply defined ridges of unpigmented soft tissues and are arranged in a well-marked pattern, the chief features of which are a medial band of narrow striations across the nape and continued down on each cheek immediately behind the eyes, an interorbital band of similar striations continued down the sides of the head in front of the eyes, and a pair of rostral bands, one on each side, stretching as a curved series of wider ridges from above each posterior nostril towards the snout. There are also a number of minor groups of these striations present as shown in the figure. There is a large posterior nostril and a somewhat smaller anterior one, with a very small pore between them which also seems to lead into the nasal cavity. The peculiar pair of pores at the end of the snout, interpreted by Borodin as anterior nostrils, are probably the terminal pores of the supraorbital branches of the lateral line system and the pore above and slightly behind each anterior nostril would also seem to belong in this series, while the pore immediately

below the anterior nostril and the one farther back at the lower margin of the nasal cavity are undoubtedly connected with the infra-orbital branch of the lateral line system on the head.

Genus **GIBBERICHTHYS** Parr

Gibberichthys Parr 1933, Bull. Bingham Oceanogr. Coll. Vol. III, Art. 6. p. 5.

The capture of a much larger specimen of this genus, which was previously known only from a single small specimen about 32 mm. long without caudal fin, makes it possible to add several new features to the generic diagnosis. Most significant of the newly discovered characters is probably the fact that the squamation, which is complete on trunk and tail and consists of thin, but not excessively thin, cycloid scales, is entirely subcutaneous, i. e., the scale pockets are completely closed and covered by a thin, generally transparent, continuous sheet of epidermis without openings of any kind. The papillate lateral line organs arise from this external cuticle, 6–8 in a vertical row across the middle of two vertically adjacent scales.[22] Head without scales, all ridges and crests spinulose, as previously described. A second important feature is found in the character of the 5 anterior dorsal and the 3 anterior anal spines. These spines are very strong and entirely rigid, the proximal portion of each being flattened and broadened to form a sharply gabled basal plate which is contiguous to the basal plates of the adjacent spines anteriorly and posteriorly. Whether these structures should be interpreted as scutes modified into spines or spiny fin rays modified into spiny scutes is, of course, only a matter of conjecture on which the author has no definite opinion, although their derivation from spiny fin rays is perhaps the most plausible interpretation of their origin. Behind these fixed spines follows one simple movable spine of the ordinary type both in dorsal and in anal fin. In the much smaller type specimen of *G. pumilus* the movable anal spine is still so soft and unclearly differentiated from the following soft fin rays that it was originally counted and drawn as one of these. In the larger specimen both the dorsal and anal movable spines are stiff and well differentiated.

The posterior nostril of the large specimen has a long, pointed, triangular flap arising from the lower half of its anterior border. In the smaller specimen this flap is only indicated by a short, pointed projection not mentioned in the previous description. As developed in the adult, this flap is undoubtedly a significant distinctive character.

In the adult the very wide pores of the younger specimen are relatively reduced and are no longer conspicuous by any unusual size compared with the pores of other Beryciformes (see figure 11). In all other respects the original generic diagnosis remains unmodified.

[22] Looking from the exterior as if they were one single greatly enlarged lateral line scale, their separation being obscured by a lateral-line streak of pigmentation in the covering epidermis.

Gibberichthys pumilus Parr

Gibberichthys pumilus Parr 1933, Bull. Bingham Oceanogr. Coll. Vol. III, Art. 6, p. 5, fig. 1.

Length without caudal fin 91 mm. Proportions in per cent of length without caudal fin: Greatest depth of body 29.1. Length of head 42.3. Length of lower jaw 26.4. Length of maxillary 24.7. Length of snout 15.4. Interorbital width 17.0. Diameter of eye 6.7. Depth of caudal peduncle 10.5. Length of caudal peduncle from base of anal fin to base of middle caudal rays 23.1. Snout to ventral fins 49.5. Snout to anal fin 59.3. Snout to first dorsal spine (scute) 51.0. Snout to first soft dorsal fin ray 65.4. Length of pectorals 17.0. Length of ventrals 9.3. Length of caudal fin lobes 20.6. Length of middle caudal rays 10.5.

D. V + I + 9. A. III + I + 8. P. 15. V. I + 5. C. 19, with 7 procurrent spines above and 6 below. Twenty-eight scales in a longitudinal series under ("in") the lateral line. Four scales above the middle of the lateral line organs and about 15 below to the origin of anal fin. 6/15 long gill rakers.

The above-recorded measurements differ from those of the type specimen (31.5 mm. long exclusive of caudal) only in regard to proportions in which such differences might be expected by a comparison with a specimen of so much smaller size (head, eyes, and depth of body relatively smaller in the adult specimen here reported upon). All pectoral rays except the upper and lower two, all dorsal and anal soft rays except the first one in each fin, and all caudal rays except the upper and lower one (17 rays) are branched. Both the fixed and the movable spines in dorsal and anal fins are free from the membrane connecting the soft rays. The scales are obscured by dark pigmentation on the external epidermis in the area covered by the pectoral fin when applied to the body, in a narrow, oblique belt from the bases of the pectorals to the axils of the ventrals, in a band along the bases of dorsal and anal fin and in a narrow streak along the middle of the lateral line. Otherwise the dark pigmentation of trunk and tail is found only underneath the scales and the external transparent epidermis, producing the effect in the preserved specimen of purplish black seen through a slightly opalescent film. Head and fins uniformly black. The pores on the head are relatively much smaller in the adult than in the juvenile type and the superficial skeletal parts more spinulose, with the preopercular spines of the juvenile presented only by spinulose prominences, as is also the opercular ridge and spine.

Genus **BROTULOTAENIA** Parr 1933

Brotulotaenia crassa n. sp.

Length without caudal fin 302 mm. Proportions in per cent of length without caudal fin: Length of head 11.77. Snout 3.05. Diameter of eye 2.85.

Figure 11. *Gibberichthys pumilus* Parr. Specimen 91 mm. long. Drawn by Y. H. Olsen.

Length of maxillary 6.39. Interorbital width 2.91. Snout to anal fin 27.5. Depth of body 7.4. Length of pectorals 2.75.

D. 126–127. A. 98–100. C. 6. P. 24. Br. 7.

In the general appearance of their various parts considered separately (e. g., head compared with head, gill covers with gill covers, etc.) *B. nigra* (Parr 1933, Bull. Bingham Oceanogr. Coll. Vol. III, Art. 6, p. 50, fig. 19) and our new species, *B. crassa*, agree so completely with each other that it is impossible to discover any structural detail in their external characters by which they can be distinguished from each other. It is only in the proportions of these various parts to each other and to the entire fish that the two species differ so conspicuously that the author feels entirely convinced of their specific distinctness. Since the very elongate shape of these fishes somewhat obscures the significance of the differences in their proportions, when these are expressed in per cent of the total length without caudal fin, the accompanying illustration of the types of both species, drawn to the same scale, is presented for a visual comparison which should serve to bring out the importance of the difference in relative measurements much more clearly than do the measurements themselves, or any verbal description. It is very fortunate that the two types, which are also the only specimens known of each species, have so very nearly the same total length (302 and 286 mm. respectively) that the possibility of ontogenetic changes accounting for the differences in their proportions may safely be ruled out.

In *B. crassa* the length of the head is more than 11 (11.7), in *B. nigra* less than 10 (9.5) per cent of the length without caudal fin, the actual difference between the relative lengths of the heads in the two type specimens being over 2 per cent of the length without caudal. In accordance with the larger size of the head, all parts of the head such as eyes, snout, maxillary etc. are also larger and the distance from snout to anal fin slightly greater in *B. crassa* than in *B. nigra*. Furthermore, the greatest depth of body in *B. crassa* is nearly 7½ (7.4), in *B. nigra* only about 6 (5.9) per cent of the length without caudal. Finally it has been discovered by dissection that instead of the 5 distinct, finger-like pyloric ceoca of *B. nigra*, there are in *B. crassa* only 4 or 5 very shallow and indistinct billow-like evaginations of the gut, which is very thin-walled and expanded immediately before it joins the pylorus.

Dentition, squamation, vertical fins, details of head and general appearance as in the generic diagnosis (Parr 1933, p. 48).

Maxillary reaching to vertical from posterior margin of orbit. Pseudobranchiae of 5 very small filaments each. Gill rakers represented by 4/11 spinigerous stubs. External color dusky brownish grey (conspicuously paler than the deep black of *B. nigra*). Inner lining of mouth and gill cavity, peritoneum and outer mesenteries deep black.

Type specimen No. 3205 of the Bingham Oceanographic Collection. Atlantis Station 1478.

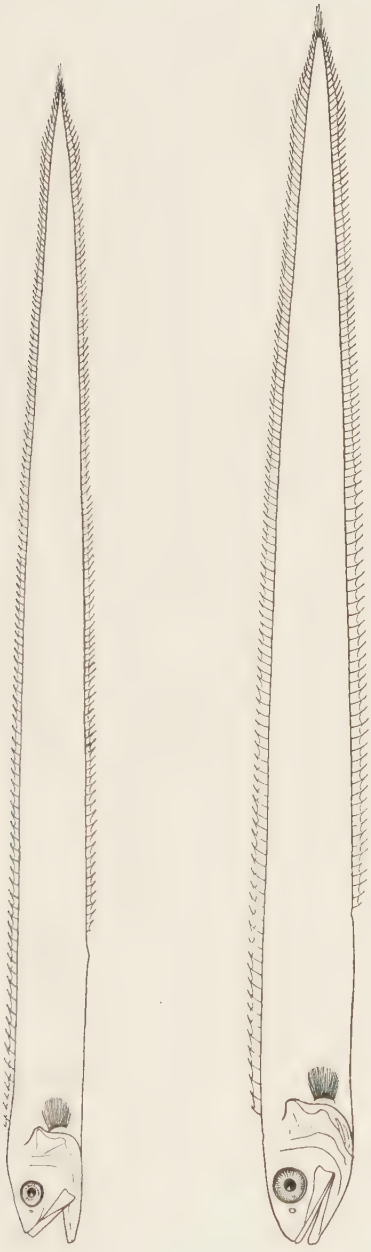

Figure 12. *Brotulotaenia nigra* Parr, 236 mm. long, above. *Brotulotaenia crassa* n. sp., 302 mm. long. Both drawn to the same scale by Y. H. Olsen.

Genus **TREMATORHYNCHUS** Regan and Trewavas 1932

With 11 known genera of female Oneirodidae and little chance of establishing the specific identity of males and females, it would seem futile to proceed with the introduction of new genera based upon the males only. Although several of its features might have been considered significant of generic distinctions, the new male described in the following has therefore been referred to the genus *Trematorhynchus*, as a convenient general designation for all male Oneirodidae. While it seems advisable to the author thus to refrain from needless duplication of generic nomenclature, the ratio of 72 known species of females to only 3 previously known species of male Oneirodidae on the other hand points to the necessity of making sharper specific distinctions among the males, since the disparity in the number of male and female species is undoubtedly in a considerable measure due to a failure to recognize specific distinctness among the males due to the inconspicuousness of their morphological differences. Thus the author is strongly inclined to question the appropriateness of grouping together under the single specific designation of *Trematorhynchus leucorhinus* Regan 22[23] male specimens with a range of variation in fin counts of D. 4–7 and A. 4–6, while the female Oneirodidae average less than 1.5 known specimens of each species.

Trematorhynchus phyllodon n. sp.

Anterior and posterior nostril contiguous, near the end of the snout far away from the eyes, which are lateral and of moderate size. Head broad, somewhat depressed, very large, and without spines. Snout square. Mouth small, transverse, horizontal, not reaching to eyes. Jaws without teeth. Snout without normal, rostral denticles but with a pair of small, wing-like bony plates diverging from bases near the median (see figure 13 C), both bones apparently somewhat damaged but with distinct indications of a series of pointed prominences along their anterior edge. No predental teeth or any other dentition in the lower jaw. Skin naked. Body short and thick. D. 6. A. 6. P. 15–16. C. 2 above + 4 branched + 2 (3?) below. Dorsal and anal fins projecting almost directly from their bases, which are only slightly forward of the external fins.

Length without caudal fin 14 mm. Total length 20.5 mm. Greatest width of skull 4.6 mm.

Skin on snout torn and partly lost. Rest of body including fin rays uniformly dark.

Type specimen No. 3206 of the Bingham Oceanographic Collection. Atlantis station 1478.

[23] 17 specimens recorded by Regan (1926, Danish "Dana" Exp. 1920–22, Oceanogr. Rep. No. 2, p. 44) + 5 specimens recorded by Regan and Trewavas (1932, Carlsberg Found. Oceanogr. Exp. 1928–30. Rep. No. 2. p. 91).

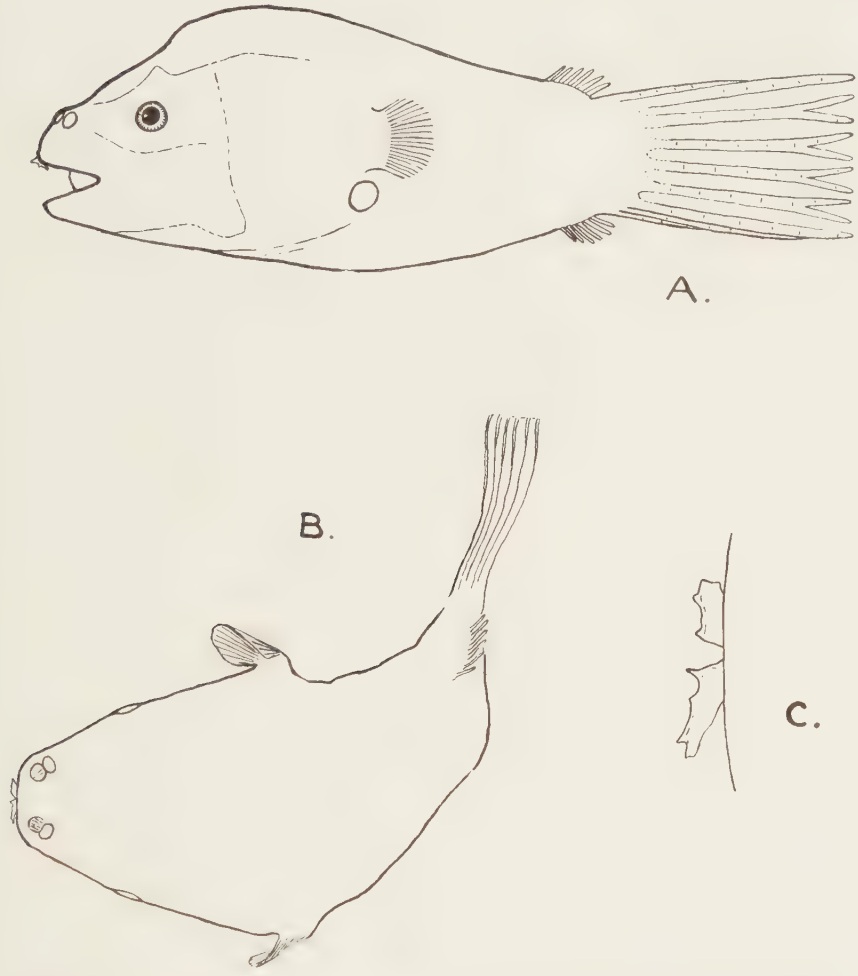

Figure 13. *Trematorhynchus phyllodon* n. sp. A. Lateral view of artificially straightened specimen. B. Natural dorsal view. C. Enlarged dorsal view of rostral denticles.

Genus **LINOPHRYNE** Collet

By analysis of the genus *Linophryne* it becomes perfectly evident that the differences upon which the distinctions between its species are based are of two entirely separate orders of magnitude and taxonomic significance, namely, (1) differences in the fundamental structural pattern of the barbel and the terminal appendages of the luminous bulb distinguishing various species singly or in

groups, and (2) variations in minor details of barbels and terminal appendages on the bulb within certain groups of species in which the fundamental structural patterns of both of these organs remain the same throughout. Thus, for instance, *L. coronata* Parr, *L. longibarbata* Borodin, and a new form to be described in the following, all agree in having a long, simple barbel ending in a small tassel of minute branches at the tip, and are also identical in the general structure of the terminal appendages on their bulbs, which consist of an anterior columnar process, more or less deeply bifurcated longitudinally at the tip, followed by a transverse pair of simple, slightly smaller columns, with a single small fin-like projection behind. Opposed to these similarities of structure are only minor differences in such features of detail as the depth to which the anterior columnar appendage on the bulb is divided, the length of the barbel and small variations on the general pattern of its tassel. Similarly *L. arborifer* Regan and *L. eupogon* Regan and Trewavas both have the same trifid terminal appendage on the bulb and both have a much branched barbel. Under these circumstances a simple subdivision of the genus into a number of equivalent species, only, is clearly inadequate to express the actual existence of two distinct degrees of similarities and probable relationships among the species. To achieve a more natural systematic expression of these facts, two ways of procedure are open, (1) to arrange the species into subgenera according to the fundamental structure of their bulbs and barbels, or (2) to treat the forms differing merely on minor points as subspecies only, of a species defined by their common characteristics. The latter procedure has been adopted here for two reasons. In the first place, it does not unnecessarily multiply the nomenclature by the introduction of new designations. Secondly, only 3 species of *Linophryne* are known from 2 specimens each, the rest being known from single specimens only. Since clearly every new specimen cannot also be a new species, there is therefore considerable reason to suspect that many of the differences in minor details may prove to represent individual variations only, leading to an ultimate reduction of the present nomenclature, and in realization of this possibility an elevation of the groups to higher ranks would be illogical.

On the basis of these considerations, the author will propose the following synoptic rearrangement of the genus *Linophryne*.

Synopsis of the species of *Linophryne*

Inadequately known forms:

1. *Linophryne arcturi* (Beebe 1926).[24] Barbel long, undivided (figure shows minute filaments arising from its distal portion). Bulb on illicium with a long, black, simple terminal column changing abruptly into a thin, colorless, distal filament. Description inadequate.

[24] *Diabolidium arcturi* Beebe: Bull. N. Y. Zool. Soc. Vol. XXIX, No. 2. March–April, 1926, p. 80, with figure.

2. *Linophryne bicornis* Parr 1927.[25] A strong, tapering, simple, lateral appendage arising on each side of the bulb of the illicium near its middle, with a small dermal protuberance beneath each appendage on the proximal portion of the bulb. Barbel and distal portion of bulb lost in the type. The introduction of a new species on the basis of such a damaged specimen was perhaps unfortunate, but the species still remains distinct from any other described form of *Linophryne* by the features of the preserved portion of its bulb.

I. Processes and appendages on the bulb of the illicium not confined to its distal surface only, but also arising in one or two lateral series from its middle and usually also its proximal portions.

A. A single series of numerous simple (?) filaments on each side of bulb. Barbel split into a bundle of (about 16) equal, simple, thin branches arising together from a short basal peduncle.... *L. polypogon* Regan

B. Bulb with a series of 6 or 7 branched filaments on each side. Barbel slender, simple, but for a short series of minute branches forming a very small tassel at its distal end... *L. racemifera* Regan and Trewavas

C. Bulb with a double row of branched appendages on each side and a simple, short, tapering filament at its distal end. Two branches arising in sequence in the proximal part of the distal three-fifths of the short barbel. Main stem beyond the last branch with a minute filament and a single series of stalked to sessile minute tubercles. Similar tubercles also in an irregular series on the proximal branch and in a single pair about the middle of the second branch.

L. brevibarbis Parr

II. Processes and appendages on bulb of illicium confined to its distal surface only.[26]

A. Bulb with only a single, simple, distal filament. Barbel bearing a lateral branch and then bifurcating; each terminal branch with distal filaments..... *L. macrodon* Regan

B. Bulb continued into a stout terminal process[27] bearing a bundle of

[25] *Linophryne bicornis* Parr: Bull. Bingham Oceanogr. Coll. Vol. III, Art. 1, 1927, p. 9, fig. 2.

[26] Without extending beyond the distal hemisphere of the bulb the appendages in this group may reach farther down in the posterior median line than they do laterally, where they are never found in series or single processes extending to or beyond the equatorial region of the bulb as they always do in group I.

[27] Judging from the figure (Regan and Trewavas 1932, fig. 167, p. 109) there can hardly be any doubt that the distal three-fifths of the organ regarded by Regan and Trewavas as simply a part of the bulb is rather comparable with the parts designated as terminal appendages in other species. With bulb is here only understood that portion of the entire luminous organ which is characterized by the internal globular

distal filaments. Other appendages beside and behind this terminal process. Barbel short, slender, trifid distally, with simple branches.
L. argyresca Regan and Trewavas

C. Bulb with a stout terminal process carrying 3 short filaments, but with no other appendages. Barbel consisting of 3 short stout branches, each with a single series of tubercles or stout tentacles.
L. brevibarbata Beebe

D. Bulb with only two minute distal filaments. Barbel bifid, divided distally into two pointed blades, each with a series of white tubercles.
L. lucifera Collet

E. Bulb with an anterior pair of long, fringed, filaments, a median short papilla bearing 3 small filaments, two long simple lateral filaments on each side, and a similar filament behind, all arising from its distal surface. Barbel very short, dividing into a group of small branches in its distal third.....*L. corymbifera* Regan and Trewavas

F. Bulb with a single trifid terminal appendage consisting of a long tapering median filament and two shorter lateral lobes arising together from a short basal peduncle. Barbel much branched almost from its base.....*L. arborifer* Regan
1. Terminal appendage on bulb about twice the length of the bulb itself. Barbel as long as fish.....*L. arborifer arborifer* Regan
2. Terminal appendage on bulb about 2/3 or less of the length of the bulb itself. Length of barbel to end of terminal filament only about 1/3 of the length without caudal fin.
L. arborifer eupogon Regan and Trewavas

G. Terminal appendages on bulb composed of a stout anterior bifid process, followed by a pair of somewhat shorter, thick, finger-like or tapering simple appendages, with a small pointed, fin-like median process behind. Barbel slender, with a small tassel of minute branches at its distal end but otherwise unbranched..*L. coronata* Parr
1. Terminal appendages white, shorter than bulb, bifid distal points of anterior process fairly large, about 1/3 of the total length of the entire appendage. Bulb nearly as long as stem. Barbel shorter than body. Lower jaw about 2/5 of length without caudal.....*L. coronata coronata* Parr
2. Terminal appendages pigmented, slightly shorter than bulb, anterior process deeply split (2/5 to 1/2 of its length). Bulb much shorter than stem. Barbel nearly twice as long as body

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body at the end of the illicium, while any part arising distally from this is considered in the group of terminal organs or appendages.

(exclusive of caudal fin). Lower jaw about 1/2 or more of length without caudal. . . . . *L. coronata longibarbata* Borodin

3. Terminal appendages white, much longer than bulb, bifid points on anterior process in the form of two minute lobes occupying only about one-eighth of the entire length of the appendage. Bulb much shorter than stem. Barbel nearly 3 (2¾) times as long as body without caudal. Lower jaw about 1/2 of length without caudal.

L. coronata diphlegma n. subsp.

Linophryne arborifera Regan

Linophryne arborifer Regan 1925: Ann. Mag. Nat. Hist. Ser. 9, Vol. XV, p. 564.

The reasons for combining *L. arborifer* Regan and *L. eupogon* Regan and Trewavas as subspecies under the common specific designation of *L. arborifer* have already been mentioned on page 42 and will be further apparent from the preceding key. It might also be pointed out that both "subspecies" have been recorded from the North Atlantic only.

Linophryne arborifera arborifera Regan

Linophryne arborifer Regan 1925: Ann. Mag. Nat. Hist. Ser. 9, Vol. XV, p. 564; 1926: Oceanogr. Rep. No. 2, Danish Dana Exp. 1920–22, p. 24, pl. III, fig. 1.
Linophryne arborifera Regan and Trewavas 1932: Carlsberg Found. Oceanogr. Exp. 1928–30. Rep. No. 2, p. 111.

In regard to the structure of the barbel in this subspecies our information is still very inadequate although the original description of this organ: "barbel as long as fish, much branched, the ultimate branches being filamentous" (Regan 1925; 1926) has been somewhat expanded by Regan and Trewavas (1932) in the statement: "barbel as long as fish, with several much branched translucent branches from a short blackish peduncle; posterior branch strongest and longest, distally divided into two main branches; ultimate branches filaments, with series of swellings." Apart from the stated length of the barbel, and the distal (as opposed to proximal) insertion of the largest sub-branch of the posterior branch, it will be found by comparison with the accompanying figure 14 and the following discussion of *L. arborifera eupogon* that even Regan and Trewavas's description is still on all other points merely general for both sub-species, if such they are, without any distinctively defined details which might help in their segregation.

The possibility that both the size of the barbel and the position of the main branching point of its posterior stem may be merely due to differences in the ages of the specimens compared will be fully discussed under the following sub-species:

Linophryne arborifera eupogon Regan and Trewavas

Linophryne eupogon Regan and Trewavas 1932: Carlsberg Found. Oceanogr. Exp. 1928–30. Rep. No. 2, p. 110.

Linophryne arborifer Parr 1927: Bull. Bingham Oceanogr. Coll. Vol. III, Art. 1, p. 10, fig. 3.

The species *L. eupogon* has been introduced by Regan and Trewavas solely on the basis of the description and figure of specimen No. 2029 of the Bingham Oceanographic Collection, previously given by the present writer (Parr 1927, loc. cit.) under the designation of *L. arborifer* Regan. The said specimen thus becomes the type of Regan and Trewavas' nominal species, and a more detailed discussion and illustration of its taxonomically most important feature, viz.: the barbel, may therefore be in place. In the previously published figure of the barbel of this specimen (Parr 1927, fig. 3) the artist has merely endeavored to give a general impression of its appearance and the main features of its system of branches. In the accompanying figure 14, prepared by Y. H. Olsen under the supervision of the writer, an effort has been made to trace every ultimate branch and terminal filament so as to give a more complete and detailed picture of their complex system.[28]

A pair of lateral and 2 posterior median main branches originate from the thick black basal part of the barbel in such rapid succession that they all appear to arise together from a short peduncle. Each branch in the anterior pair bifurcates transversely almost from its base, the medial sub-branch being larger than the lateral one, and both sub-branches being again more or less bifurcated at the tip. The first posterior main branch originates immediately behind the anterior lateral pair and carries a single series of 10 sub-branches along its entire anterior surface from immediately below its origin to the base of its terminal filament (11th branch). The anterior two sub-branches of the first order are rather stout and dusky in their proximal parts and again carry a single series of finer sub-branches of second order along their anterior surface on their distal portion. In the following sub-branches of the first order these second-order sub-branches are reduced to traces or filaments, and the first-order sub-branches themselves become thinner and paler in order of their sequence distally, the last 4 being entirely filamentous and closely clustered.[29]

The second (main) posterior branch of the barbel also carries a single series of 8 (or 7[30]) sub-branches beginning immediately behind the origin of the first

[28] In the effort to achieve clarity, the insertions of the subbranches on the two main posterior branches have perhaps become spread out a slight bit more than they naturally are in the specimen and a slight distortion of the density of the entire system has thus resulted.

[29] See footnote concerning the figure.

[30] Dependent on whether the 7th sub-branch (in a count of 8) is regarded as a separate sub-branch of the first order, or as a second-order sub-branch originating

Figure 14. Barbel of *Linophryne arborifera eupogon* Regan and Trewavas. Drawn from type specimen by Y. H. Olsen.

posterior main branch of the barbel. The 3 anterior sub-branches on the second posterior main branch each give rise to a lateral pair of opposed filamentous sub-branches of the second order, the most anterior sub-branch also carrying a single median filament distally to the lateral pair. The more posterior sub-branches all branch in the median only, if at all. At the base of the last (8th or 7th) sub-branch (of the single anterior series), which is considerably enlarged and produced, with a long terminal filament, the stem of the second posterior main branch forms a knee in the hollow of which 3 pairs of filamentous sub-branches are inserted close together in a double row between the last single sub-branch and the base of the terminal filament, which is long (incomplete in the specimen) and irregularly branched and beset with a few minute bulb-bearing tentacles. The ends of all ultimate filaments are apparently more or less swollen when complete. Proximal portions of the lateral and of the two posterior main branches, with basal parts of the more anterior sub-branches of these, more or less dusky; basal peduncle of barbel black.

In the type specimen which now measures 29 mm. without caudal fin the length of the barbel from the anterior base of the peduncle to the knee (the base of the last sub-branch in the single series) of the second posterior main branch is 4.5 mm. or 15.5 per cent of the length of the fish. In the Atlantis collection from Station 1478, we also find another specimen, 26.5 mm. long without caudal fin in which the length of the barbel to the knee is only 3.5 mm. or 13.2 per cent. Although their difference in size is small, there is thus a not inconsiderable difference in the relative dimensions of the barbels in the two specimens pointing to a comparatively late but very rapid growth of this organ which might easily lead to the dimensions found in the much larger type of *L. arborifera arborifera* in which the length of the barbel, terminal filaments included, equals the length of the fish which is 50 mm. without caudal fin.[31] This possibility gains further support from a comparison of the morphological development of the barbels in the two Bingham Collection specimens.

In the smaller one, collected at Atlantis station 1478, the first posterior main branch of the barbel carries only 3 small anterior, pale, branched, filamentous sub-branches followed by one transverse pair of filaments and the terminal filament of the branch itself, all occupying only the distal two-fifths of the stem of the branch, the proximal, dusky, three-fifths being as yet unbranched. On the second main posterior branch, the enlarged and produced sub-branch at the knee is only the sixth (instead of 8th as in the larger specimen) and last of the single anterior series. The barbels of the two specimens are otherwise identical. It thus appears that an increase in the number of sub-branches has also taken

from the anterior surface of the next following sub-branch of the first order near its base. The first interpretation seems the most natural one in the specimen on hand.

[31] The lengths of the barbels, terminal filaments included so far as preserved, in the two specimens of the Bingham Collection are 27.5 and 38 per cent, respectively.

place between the sizes of 26.5 and 29 mm. without caudal fin, and if this increase is continued with further subdivisions and development of filaments to size 50 mm. without caudal, it is quite conceivable that the barbel found in the type of *L. arborifer arborifera* might result.

It is thus perfectly possible, or even probable, that further investigation may serve to confirm the identification of the type of *L. arborifera eupogon* first suggested by the writer, and in the meantime it would certainly seem unwarranted to give more than subspecific significance to the features shown by the smaller specimens.

Linophryne coronata Parr

Linophryne coronata Parr 1927: Bull. Bingham Oceanogr. Coll. Vol. III, Art. 1, p. 13, fig. 4.

Linophryne longibarbata Borodin 1930: Proc. New England Zool. Club XI, p. 87.

Including the example obtained by the "Atlantis" at station 1478, three specimens are now on record which agree with the type of *L. coronata* in all general features of their bulbs and barbels and in the system and structure of the

Measurements of *Linophryne coronata* Parr

Subspecies	*longibarbata*	*coronata*	*diphlegma*
Length without caudal fin in mm.	75.5	33[32]	33
Proportions in per cent of length without caudal:			
Length of caudal fin	42	40	44
Length of maxillary	53	38	51/54
Length of lower jaw	56.3	39	51
Top of head before base of sphenotic spine to lower posterior corner of lower jaw[33]	59.5	50	60.6
Snout to posterior base of sphenotic spine	29	29	28.8
Snout to eye	24	18	21
Interorbital skeletal width	10	12.5	13
External width between bases of sphenotic spines	28	31	32
Snout to gill opening	62	52	56
Diameter of eye	3.3	3.6	4.5
Preopercular to sphenotic spine	44	39.4	45.5
Angular to preopercular spine	21	18.8	20
Longest lower fang	18.2	9.4	21
Longest upper fang	11.3	4.9	13
Total length of barbel	187	90	276
Length of tassel	28	11.5	9
Length of illicium stem	17.5	10.0	22.7
Length of bulb	9.5	9.1	10.6
Length of anterior terminal process on bulb	8.8	7.0	18.0

[32] The measurement previously recorded has reference to the point at which the thick black skin of the body changes abruptly into the pale caudal fin membrane.

[33] Angular spine not included.

branches and appendages on these parts, differing only in such details of secondary importance as mentioned in the key on page 44 and discussed and illustrated in the following. Since it is obviously possible, with so little material available, that these minor differences may subsequently be found to be only a matter of individual variations, and since the characters in question are under no circumstance of the order of importance of those on which our specific distinctions within the genus have otherwise been based (see page 42), the three specimens are here tentatively recorded as subspecies only, of *L. coronata*. As in the case of *L. arborifera*, the three "subspecies" have all been recorded from the same general region of occurrence, in this instance confined to the western North Atlantic only.

Terminal appendages on the bulb of the illicium composed of a stout bifid process anteriorly; followed by a pair of shorter, simple, thick, finger-like or tapering appendages; with a small, pointed, fin-like median process behind. Barbel slender, with a small tassel of minute branches at its distal end, but otherwise unbranched.

Tassel with a circle of four equal, small branches, at its base (proximal end), each with a uniserial row of stalked or sessile bulbs on one side, a simple or branched, minute appendage or filament on the other, and a simple terminal filament with swollen tip at the end (see figs. 15–20). Beyond these basal branches of the tassel the main stem of the barbel immediately branches once or twice, in a different manner in each subspecies.

Linophryne coronata coronata Parr

Linophryne coronata Parr 1927: Bull. Bingham Oceanogr. Coll. Vol. III, Art. 1, p. 13, fig. 4. Regan and Trewavas 1932: Carlsberg Found. Oceanogr. Exp. 1928–30 Rep. No. 2, p. 110.

Figure 15. *Linophryne coronata coronata* Parr. Drawn by Y. H. Olsen.

This subspecies differs conspicuously from the other two subspecies by its relatively much shorter jaws and fangs as one may see from the measurements given on page 49 and from a comparison of the accompanying figure 15 with figures 17 and 19; the jaws being only about 2/5, the longest lower fang 1/10 and the longest upper fang 1/20 of the length without caudal fin.

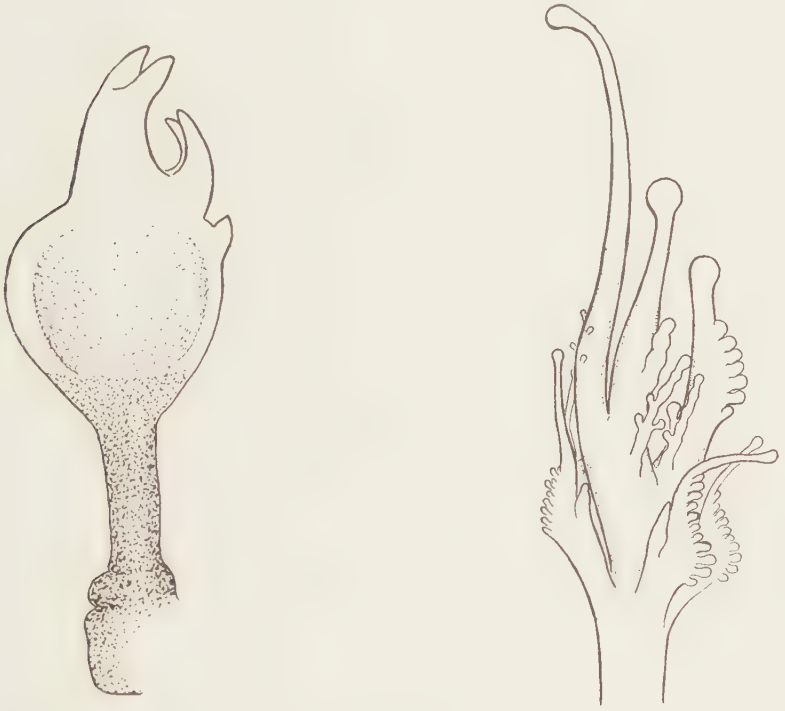

Figure 16. *Linophryne coronata coronata* Parr. Illicium left; tassel of barbel right. Drawn by author.

Beyond the ring of the four basal branches on the tassel, the main stem of the barbel immediately gives rise to two relatively short and thick branches in close succession (see figure 16) and ends in a somewhat longer, simple, terminal filament with a swollen tip. The first branch carries a small pair of rather irregular lateral appendages, with a minute median stalked tubercle between them, posteriorly at its base; a single series of 7 almost sessile small bulbs along its middle portion anteriorly; and a thick, swollen bulb at its end. The second branch also carries a pair of irregular appendages at its base, but anteriorly instead of posteriorly, and has a simple appendage farther up, but carries no

regular series of bulbs or tentacles. The second branch also ends in a greatly swollen tip. The terminal filament carries a few minute stalked tubercles on the proximal third of its length. Total length of barbel slightly less than the length of the fish without caudal fin.

The bulb of the illicium, exclusive of its distal appendages, is almost equal in length to the stem; the anterior process on the bulb is slightly shorter than the bulb itself and is divided distally for about one-third of its length. Distal processes all colorless.

Only the type known.

Linophryne coronata longibarbata (Borodin)

Linophryne longibarbata Borodin 1930: Proc. New England Zool. Club. XI, p. 87; 1931, Bull. Mus. Comp. Zool., LXXII, p. 83; Regan and Trewavas 1932: Carlsberg Found. Oceanogr. Exp. 1928–30. Rept. No. 2, p. 107.

So far the structure of the appendages and branches on the illicium and barbel of this form have neither been figured nor described, nor has a general illustration of the species been published. To supply this want, the author has examined the type and prepared the accompanying diagrammatic figures of the specimen to which the following description may be added.

Length of jaws about 1/2 or more; longest lower fang nearly 1/5 and longest upper fang more than 1/10 of length without caudal fin.

Main stem of barbel bifurcating opposite terminal filaments of the four proximal branches in the tassel. One branch trifurcates a short distance beyond the bifurcating point; the other gives rise to 3 sub-branches in a group around a terminal continuation of the branch itself, which latter again gives rise to an opposed pair of smaller sub-branches (trifurcates) from its proximal portion. The middle and longest of the three sub-branches arising simultaneously from the second main branch of the barbel, as above described, carries two series of minute filaments with swollen tips on its proximal portion, the other ultimate branches only one single series each beyond which they all end in a terminal filament with swollen tip. A short distance in advance of the tassel the barbel carries a soft, black outgrowth covered by normal skin. Total length of the barbel almost twice the length of the fish without caudal fin.

Bulb of illicium exclusive of distal appendages only slightly more than half the length of the stem; anterior process on bulb slightly shorter than bulb itself, divided almost to its middle. Undivided portion of anterior process and middle pair of terminal appendages, except along their posterior margins, black. Distal half of bulb, posterior fin-like process, posterior margins of paired appendages, and divided tips of anterior process white.

Only the type seen by the author. Second specimen recorded by Borodin (1931, p. 83) not available for examination.

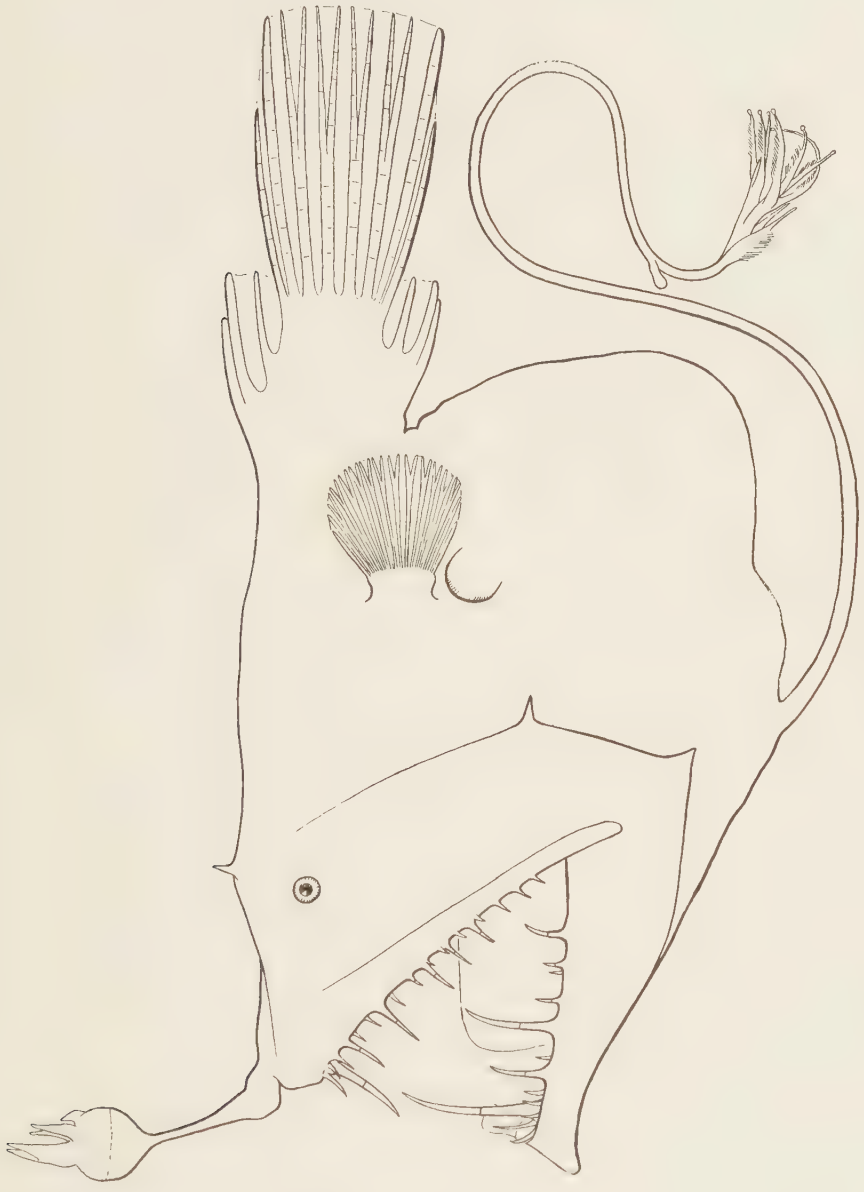

Figure 17. *Linophryne coronata longibarbata* Borodin. Drawn by author and Y. H. Olsen.

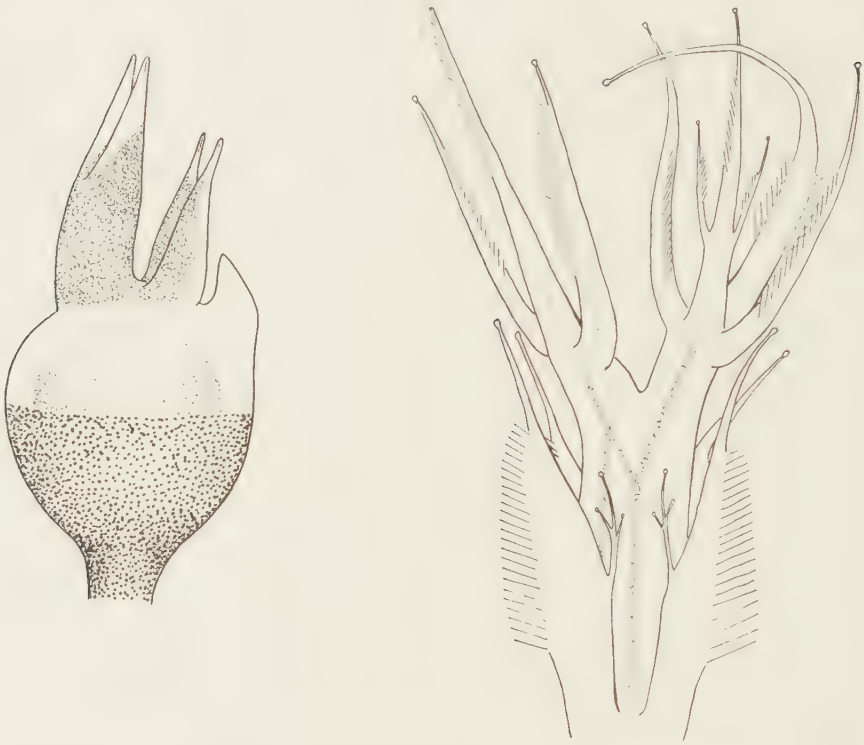

Figure 18. *Linophryne coronata longibarbata* Borodin. Bulb of illicium left; tassel of barbel right. Diagrammatic sketches by author.

Linophryne coronata diphlegma n. subsp.

The proportions of the type specimen are given in the table on page 49. It agrees with *L. coronata longibarbata* in the length of the jaws and fangs; the jaws being about 1/2, the longest lower fang about 1/5, and the longest upper fang about 1/8 of the length without caudal. It also agrees with subspecies *longibarbata* in the proportions between stem and bulb on illicium, but differs from both *longibarbata* and *coronata* in the extreme length of the barbel and in the length and appearance of the anterior terminal process on the illicium.

The main stem of the barbel bifurcates between the bases of the four proximal branches in the tassel. One main branch gives rise to a small lateral filament from its distal third and ends in a thick, fist-like swelling apparently distended by numerous internal bulbs. The other main branch bifurcates again, one of the sub-branches being simple with a short series of small sub-tentaculate

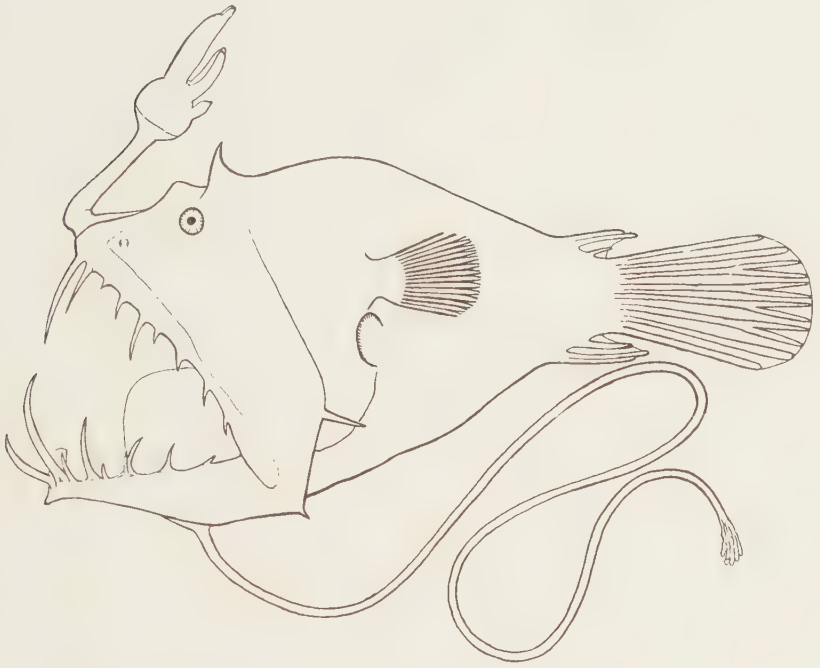

Figure 19. *Linophryne coronata diphlegma* n. subsp. Drawn by Y. H. Olsen.

bulbs and a terminal filament with swollen tip; the other sub-branch being twisted upon itself so that it is difficult to disentangle its profusion of long-stemmed bulbs or bulb-bearing filaments all arising from the same side, but whether in a single series or a more irregular arrangement is not clear. Some of these filaments appear to be branched at least once, with swellings at the end of each branch.

The anterior process on the bulb of the illicium is very long, about $1\frac{2}{3}$ times the length of the bulb itself, while its bifid tips are reduced to a pair of short protuberances only about 1/7 of the length of the undivided portion, and well marked off from the latter in their outlines. The paired terminal appendages are also relatively elongate with distinctly mammilliform tips. Terminal appendages entirely colorless.

Only the type known. No. 3207 of the Bingham Oceanographic Collection. Atlantis station 1478.

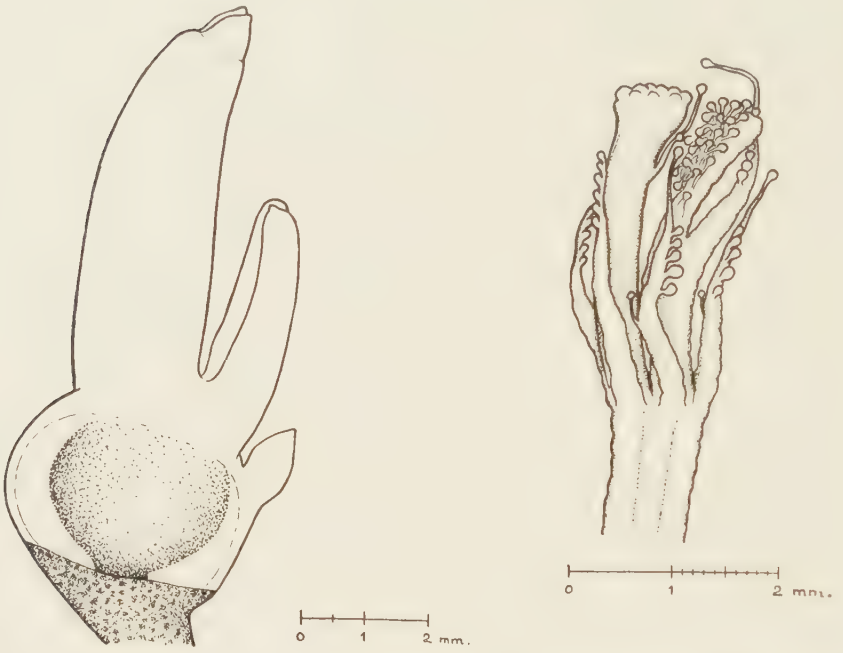

Figure 20. *Linophryne coronata diphlegma* n. subsp. Bulb of illicium left; tassel of barbel right. Drawn by author

GENUS **BOROPHRYNE** Regan

Borophryne masculina n. sp.

D. 3. A. 3. P. 16. C. 2 + 4 + 2. Head in the shape of an oblique pyramid resting on its smallest side, the tip of the lower jaw forming its apex. A tooth-bearing rostral prominence above and in front of the upper jaws which are toothless. The lower jaw projects forward of the upper and of the rostral prominence. Nasal region somewhat swollen. Anterior nostril only about half the diameter of the posterior nostril, which is large and situated close to the eye. Eyes fairly large, lateral, directed outward, not telescopic. A long, slender supraorbital (sphenotic?) spine directed outwards at right angle above and behind each eye. Lateral line organs on short pigmented tags connected by thin white lines. Dorsal and anal fin rays projecting through the skin with their tips, only, near the base of the caudal fin far behind their own fin bases.

In all of the characters mentioned in the preceding the specimen agrees perfectly with the descriptions and figures given by Regan and Trewavas (1932, Carlsberg Found. Oceanogr. Exp. 1928–30. Rep. No. 2, p. 18, figs. 7

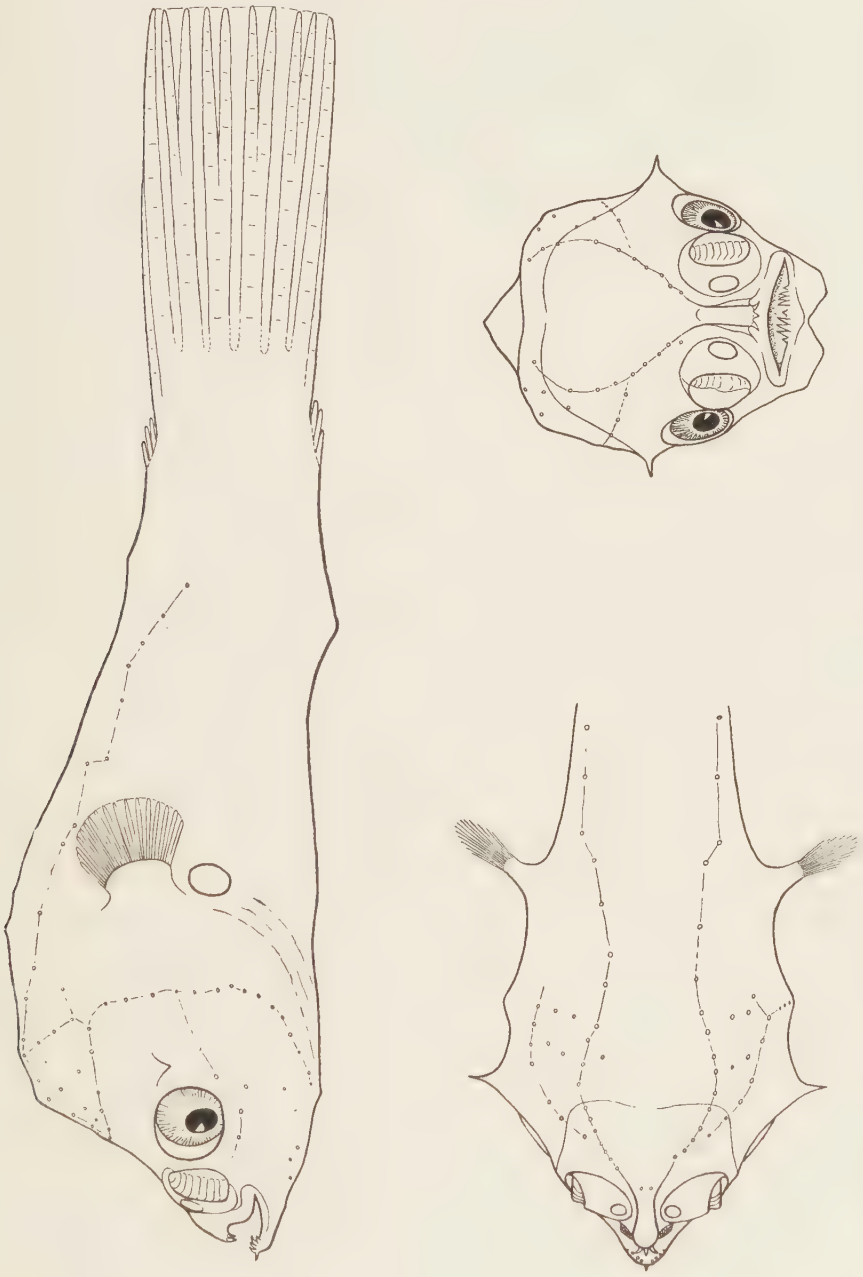

Figure 21. *Borophryne masculina* n. sp. Lateral view above; dorsal view of head at lower left; frontal view of head at lower right. Drawings by Y. H. Olsen.

and 8, and p. 106) of the two males found attached to two female representatives of the genus *Borophryne*, and it is thus for the first time possible to introduce a new Ceratioid species referred to its proper genus on the basis of a still unattached male only. The characters on which the specific distinction of *B. masculina* from *B. apogen* is based all have reference to minor details, only, and do not involve any differences which would even approach to generic significance. It is, on the contrary, not entirely without hesitation that the new form has been given the rank of a separate species rather than that of a subspecies only. The differences are certainly not greater or more significant than the differences between the two males referred by Regan and Trewavas to a single species on the basis of their identification of the females to which the males were attached. At this point, however, the author would like to raise the question as to whether the identification of two similar-looking females is at all justified in the face of such conspicuous differences in the males. The features of one sex must certainly, until otherwise proven, be considered to be equally as important as the features of the other sex, and it hardly seems reasonable to regard as specifically identical one completely pigmented and one entirely umpigmented male which also show differences in other respects.[34]

Borophryne masculina differs from both of the male specimens referred by Regan and Trewavas to *B. apogon* by having only 3 rostral denticles, instead of 5. It differs further from the pigmented form in having the ends of 3 dorsal and 3 anal rays projecting freely through the skin at the base of caudal fin; and from the unpigmented form by being completely pigmented and by the fact that the base of dorsal fin is well behind the base of anal, the dorsal fin rays being correspondingly shorter.

Lower jaw with 4 small teeth on each side near the median in which a small single tooth projects forward. Three rostral denticles, one median and one on each side. Eight nasal lamellae. Nasal cover unpigmented except along its base and around the rim of the posterior nostril. Nape high and somewhat sharp behind the skull, probably supported by spinous processes. Lateral line system complete on head, incomplete on body (see figure 21). Body somewhat compressed.

The type specimen is a male measuring 24 (16 + 8) mm. The distance from the tip of the rostrum to the gill openings equals about 45 per cent of the length

[34] In the pigmented male Regan and Trewavas mention 2 dorsal and 2 anal rays projecting with their ends through the skin at the caudal base. In the unpigmented male 3 dorsal and 3 anal rays are recorded, and it seems indicated in the illustration that all three rays of each fin must project. There thus appears to be an actual difference between the two specimens with respect to the number of rays projecting from the skin, and it is also probable that only two rays altogether in each fin may be found upon dissection of the pigmented male. Regan and Trewavas (loc. cit., p. 19) also mention differences between the two specimens in regard to the projection of the lower jaw.

without caudal fin, the greatest width of the head exclusive of the sphenotic spines about 35 per cent, the greatest depth about 37 per cent, the diameter of the eyes 7.5 to 8 per cent, the length of lower jaw 21.5 per cent, and the distance from the tip of the rostrum to the eyes about 13 per cent. Other proportions as shown in the illustration, which is based upon camera lucida drawings except in regard to the caudal and pectoral fins which are curled up in the specimen.

Entire specimen, including caudal and pectoral fin rays black, with the exception of the nasal cover only (see above).

Type specimen No. 3208 of the Bingham Oceanographic Collection. Atlantis station 1478.

Contribution No. 36

Reprinted from The Biological Bulletin, Vol. LXVI, No. 1, February, 1934

THE CONTROL OF THE DERMAL MELANOPHORES IN ELASMOBRANCH FISHES

GEORGE H. PARKER AND HELEN PORTER

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Introduction

The color changes in the elasmobranch fishes have received scant attention. Aside from the negative results recorded by Schaefer (1921) for *Raja clavata* and *Raja batis,* the only account of this subject known to us is that given by Lundstrom and Bard (1932) in a paper devoted to the action of the pituitary secretions on the color changes in the dogfish, *Mustelus canis.* In this paper it is shown in a very conclusive and adequate way that hypophysectomy is followed by a permanent paling of the fish and that this paling can be changed into a temporary darkening by the injection of pituitary extract. It is further shown by these authors that the changes thus experimentally induced agree in general with those normally excited in this fish by alterations in the tint of the background.

In their account of these changes Lundstrom and Bard give a very full description of the alterations of the fish from light to dark, but the reverse change is passed over with very slight comment. Although no specific statement is made by them on this point, it seems probable from the contexts in their paper that they regard the light phase of the dogfish as due to a decrease in the amount of the pituitary secretions in the fish's blood or possibly to a complete absence of these secretions from this fluid. According to them, this condition is in contrast to that in which there is an abundance of these secretions which thus induces the dark phase in the fish. That this dark phase is due to pituitary activity seems to us adequately and sufficiently shown and in this respect our own results fully agree with those of Lundstrom and Bard, but that the light phase is the consequence of the mere absence of the pituitary secretions appears to us quite unlikely as the observations recorded on the following pages tend to prove. Our own work was carried out on the smooth dogfish, *Mustelus canis,* partly at the Woods Hole Oceanographic Institution and partly at the Marine Biological Laboratory. To the Directors of these two establishments we wish to express our sincere thanks for the facilities and materials supplied us by which our investigations were made possible.

[1] Contribution No. 36.

Changes in the General Coloration of the Dogfish

When a light-colored dogfish is placed in a black-walled tank illuminated from above, the fish quickly becomes dark. The time required for this change is from half an hour to an hour or two, and the subsequent retention of the fish in the dark-walled aquarium does not deepen further its tint.

Whether of light or dark shade in the beginning, dogfishes from which the eyes have been removed become quickly dark and remain indefinitely so irrespective of the shade of their surroundings. Such blinded fishes attain as a rule a deeper gray than the maximum shown by the normal fishes in a dark-walled tank. In fact, the darkest dogfish that we have ever seen was one whose eyes had been removed.

Dark dogfishes placed in an illuminated white-walled tank become gradually very light. Occasionally under such circumstances they fail to reach the extreme of light coloration and will maintain even in fully light surroundings a gray tint. This failure of full response has also been noticed by Miss Lundstrom, who called our attention to the frequent occurrence of cataract-like obscurities in the dioptric portion of the dogfish eye and suggested these defects as a possible explanation of this failure on the part of certain dogfishes to turn fully light. Unfortunately we were unable to test this suggestion. The majority of dogfishes, however, become extremely light in light surroundings and remain so. This paling is often associated with a pink tone that gives to the fish the appearance of a delicate blush. Such a coloration is doubtless brought about by the subdermal blood-vessels, which in consequence of the translucency of the skin in the light state show through this covering and thus affect the general tint of the fish.

The establishment of the light phase in the dogfish is a relatively slow process. A dark fish when put in a white-walled tank grows light at first rapidly and then very much more slowly till the maximum lightness is reached. This maximum is attained only after two or more days whereupon the fish remains indefinitely pinkish white.

What in general is thus slowly accomplished in a white-walled tank may be reached quickly by a subcutaneous injection of adrenalin chloride (Parke, Davis and Company). About five minutes after a dogfish has received a hypodermic injection of 0.5 cc. of diluted adrenalin, 1 part in 10,000, a general paling of the skin can be seen and half an hour later the fish is fully light. The degree of paling, even when 1 cc. of adrenalin is introduced into the fish, is seldom as pronounced as that seen in fishes from the white-walled tank, but occasionally the injected fishes reach what in our experience is the maximum of light coloration.

It is comparatively easy to show by placing the pectoral fin of a dog-

fish under the compound microscope that the dark phase of the fish is due to the expansion of the dermal melanophores and the light one to their contraction. Excellent figures of these conditions have already been published by Lundstrom and Bard (1932).

Fin Bands

If in a moderately dark dogfish deep cuts transverse to the rays of any fin are made or similar cuts are inflicted on the body, these cuts give rise to light bands or splotches which are clearly visible in the skin for many hours or even days thereafter. If the spinal cord of a dogfish is transected in the posterior part of the body, the portion of the fish behind the cut becomes irregularly slightly lighter. The light bands are best seen when they are produced on the pectoral fins and are most easily induced by making a well circumscribed cut completely through the fin from one face to the other at right angles to the rays. Such an incision is conveniently made by means of a chisel with a cutting edge of about one centimeter.

In the pectoral fin the course of the main blood-vessels can be easily seen through the translucent substance of that organ and the cut may be made either distal or proximal to the chief vessels, thus leaving the blood supply to the fin essentially undisturbed. The cut necessarily severs a number of the smaller vessels, but, if its position is well chosen, it can be shown to introduce no serious interference with the circulation. After such a cut has been made a light band quickly develops extending from the cut to the free edge of the fin. This band assumes the width of the cut, never more, and always extends over the fin distally from the cut, never proximally. It follows very closely the lines of the fin rays. Its detailed nature and fate depend upon the light or dark condition of the dogfish in which they are induced.

In a dark dogfish the band is a lightish area of irregular outline (Fig. 1). It is seen with certainty in from ten to fifteen minutes after the cut has been made and it reaches its maximum in about a day. After this it gradually fades out, to disappear completely in from two to three days. A narrow band three to four millimeters wide will, however, disappear in a little over a day; one of a centimeter wide will remain visible for as much as two to three days. The light band fades by being replaced with the dark coloration of the surrounding fin area, the center of the band near the periphery of the fin being the last to disappear (Fig. 2).

When a cut is made in the pectoral fin of an eyeless dogfish, a light band also appears in about a quarter of an hour and then follows the usual course of disappearance.

In a light dogfish the band is much more clearly defined in outline

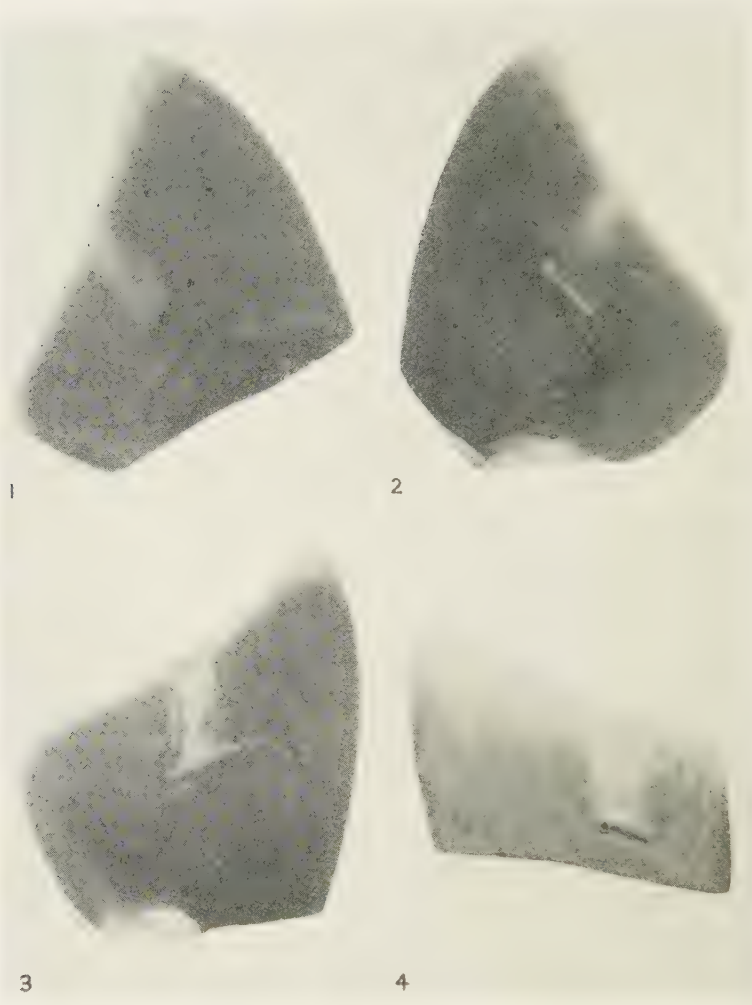

PLATE I

Dorsal View of Pectoral Fins from the Dogfish, Mustelus canis

FIG. 1. A fin from a dark dogfish, showing a light band which appeared as a result of an incision transverse to the direction of the fin rays and about a centimeter and a half from the edge of the fin. The figure represents the state of the band about an hour after the cut had been made.

FIG. 2. A fin from a dark dogfish, showing a light band in process of gradual disappearance. The band was produced by the same kind of a cut as that shown in Fig. 1, but in the course of two days it became partly obliterated by the invasion of the general dark coloration.

FIG. 3. A fin from a dark dogfish, showing a light band in process of disappearance, an operation here locally checked by a longitudinal cut on one side of the band.

FIG. 4. A fin from a light dogfish, showing a light band several days after the initial transverse cut had been made.

than in a dark one. Its edges are sharply marked and they can be traced from the ends of the cut to the edge of the fin (Fig. 4). Although the fish may be extremely light in color, the band is always still lighter and agrees in tint with the white border of the fin. Unlike the light band on the dark fish, that on the light fish seems never to be obliterated. At least in all light dogfish kept by us in the laboratory tank the light band has persisted as long as the fish has lived, a period of at most about five days. When from a light dogfish with a light band the eyes are removed the fish darkens and the band eventually disappears as it does in an ordinary dark fish in which it had been induced by a cut.

Discussion

We are entirely in accord with Lundstrom and Bard in holding the opinion that the dark phase of the dogfish is due to the action of an expanding hormone carried to the melanophores from the pituitary body by the blood. We are convinced of the correctness of this opinion partly because of the facts advanced by Lundstrom and Bard and partly because of our own results. If the blood of a dark dogfish is drawn, defibrinated, and injected subdermally into light and into dark dogfishes, it will be found to have no effect upon the skin of the dark fishes but to produce a well-defined dark area on that of the light ones. This dark area, which may measure several centimeters in diameter, surrounds the point of injection and when examined under the microscope can be shown to have been produced by the expansion of melanophores.

Another observation that supports the general conclusion under consideration was made on operated fishes. If in a light dogfish a longitudinal cut parallel with the fin rays of the pectoral fin is made near the edge of a light band and the dogfish is put in a dark-walled tank, as the light band disappears it will last be seen next the longitudinal cut and on that side of it adjacent to the axis of the original band (Fig. 3). This method of disappearance is consistent with the idea of an invading, fluid-borne, expanding hormone such as has been claimed by Lundstrom and Bard.

For these and other reasons we are fully convinced that the dark phase of the dogfish results from the application of a blood-soluble, expanding hormone to the dermal melanophores.

The light-colored bands and their reactions as we have described them seem to us to be equally conclusive evidence that the light phase of the dogfish is dependent upon nervous action. To be sure, these bands follow the courses of the smaller blood-vessels and might therefore be attributed to an operative disturbance in the circulation. But, as already stated, the cut can be made either proximal or distal to the chief

blood-vessels of the fin and the resulting band, which may easily be two or more centimeters long, is in large part so far from the exciting incision that the local circulation is completely undisturbed. This can be easily demonstrated by inspecting under a compound microscope the living pectoral fin of an operated dogfish. If the light ventral face of such a fin be examined, the flow of blood in the capillaries within the limits of the band can be seen to be as free and as abundant as it is in the regions outside the band. There is thus no reason to attribute the formation of the band to any circulatory irregularity, and the conclusion that is naturally arrived at is that the only other radial system, namely the nerves, is responsible for the bands.

The fin nerves run closely parallel to the fin rays and much more accurately so than the blood-vessels do. Since in light dogfishes the edges of the light bands follow very closely the courses of the rays, they must also follow the lines of the nerves. Hence we believe the light bands to be dependent upon the nerves. When a transverse cut is made in a fin a number of fin rays with their attendant nerves are severed and this severance is so vigorous a stimulus for these nerves that they excite the melanophores of their region to full contraction. The result of this response is that a light band is produced corresponding in extent to the area of distribution of the contracting nerves. Such a view coincides completely with what we know of the distribution of light spots and bands that result from skin cuts on other parts of the body of the dogfish. Spots or bands thus induced always extend from the region of the cut in the distal direction of the nerves and in no other. They are due in our opinion to the excessive activity of the severed contracting nerve fibres.

How long their contracting activity can be kept up we do not know. The bands in a light dogfish will maintain themselves essentially undiminished for as much as five days, the longest period that we have been able to keep an operated dogfish alive in the experimental tank. This period is much too short, judging from our results on *Fundulus* (Parker and Porter, 1933), to reach a condition of nerve quiescence and in our opinion the fin nerves concerned with melanophore contraction in the dogfish must be regarded as in more or less continuous action as a result of the irritation of the wound for a period of at least five days.

During this period, however, there appears to be in the dogfish a certain amount of decline in activity. This is seen in the disappearance of the light bands in dark dogfishes. As already pointed out, the light bands which are indefinitely persistent in light fishes disappear in at most two or three days in dark fishes. This disappearance we believe to be due to the action on the contracted melanophores of the expanding,

blood-borne hormone which counteracts the contracting action of the nerves. When the fin nerves are first cut in a dark dogfish they are so vigorously stimulated that their activity predominates locally over that of the expanding pituitary hormone and the light band is formed. As time goes on, however, the melanophores of the band come more and more under the influence of the expanding hormone, a step rendered possible, we believe, by the subsidence in activity of the contracting nerve fibres. Thus the disappearance of the light band from the fin of a dark dogfish depends, in our opinion, in part at least on the gradual decline in the activity of the contracting fibres whereby the expanding hormone may assert itself. As already stated, we have never seen instances among dogfishes where this decline was complete, for if a dark dogfish in which the band has disappeared is put in a white-walled tank the band will reappear as the fish lightens. Nevertheless, we believe that in time this activity might completely subside, as it has been shown to do in the expanding fibres of *Fundulus* (Parker and Porter, 1933).

It must be clear from what has been stated that in our opinion the darkening of the dogfish skin is due to the action of an expanding blood-soluble hormone on the dermal melanophores of the animal. It must also be clear that the paling of the dogfish skin results from the action of contracting nerve fibres on the melanophores, an action which when excessive may overcome that of the expanding mechanism. Do the contracting nerve-fibres produce a contracting substance or neurohumor comparable to the pituitary expanding hormone? Thus far we must answer this question in the negative and in consequence of two lines of evidence. First, when the defibrinated blood of a light dogfish is injected into a dark one, the skin of the dark individual does not lighten, showing that such blood carries no dissolved contracting neurohumor such as is stated by Meyer (1931) to occur in certain flatfishes. Secondly, if the width of a newly-produced band in the pectoral fin of a light dogfish is accurately measured and if this measurement is compared with a second one of the same band in the same place made three days later, no increase in width can be noticed. This result is opposed to the view that the light band is due to a water-soluble neurohumor produced by the contracting nerve fibres and more or less like the expanding blood-borne hormone. If such a substance were present it most certainly would induce an expansion of the band. The evidence here presented, however, does not preclude the possibility of a locally produced contracting neurohumor insoluble in blood or lymph, but it makes clear that if such a neurohumor is present, it is probably lipoid-soluble and would therefore be essentially local in action.

The general conclusion to be drawn from this discussion is that the

color changes in the dogfish are of two types, one from light to dark due to an expanding, pituitary hormone carried from the gland to the melanophores by means of the blood and other body fluids, and another from dark to light due to a contracting local action of nerves. It is interesting to note that of the many animals in which the cutting of nerves produces a change in coloration, the dogfish is the first one to be recorded in which this change is a paling. All other forms exhibit a darkening when their chromatophoral nerves are severed. This we think is due to the fact that in the dogfish, unlike other fishes, probably only contracting nerves are present and that consequently in this fish these nerves have no others with which to compete as the contracting nerves of other fishes apparently have. On stimulation these nerves in the dogfish therefore assert themselves without hindrance. The dogfish is further peculiar in that it presents a melanophore system in which the expanding action is unihormonal, modeled after that of an amphibian, and the contracting action nervous like that in most other fishes.

Summary

1. The common dogfish, *Mustelus canis,* has a dark phase and a light one due to the expansion and the contraction of its dermal melanophores.

2. The dark phase is induced by pituitary secretions carried from the pituitary gland to the melanophores by the blood and lymph as described by Lundstrom and Bard.

3. The light phase is induced through the action of contracting nerves, and is not merely the result of the absence of pituitary secretions.

4. The expanding hormone is water-soluble. The contracting one, if there be such, is apparently not water-soluble.

5. The dogfish is remarkable as the first form to be described in which the cutting of nerves induces a contraction of the melanophores and a consequent lightening of the skin.

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THE COMBINATION OF CARBON MONOXIDE WITH HEMOCYANIN*

By RAYMOND W. ROOT

(*From the Woods Hole Oceanographic Institution, Woods Hole*)

(Received for publication, November 15, 1933)

Craifaleanu (1919), on bubbling carbon monoxide gas through hemocyanin solutions, observed that they turned colorless. When exposed to oxygen, the solutions apparently failed to regain color as rapidly as when reduced with an inert gas. Consequently, he thought it likely that a carbon monoxide compound of hemocyanin was formed which is unstable in the presence of oxygen. Dhéré and Schneider (1922) also observed that hemocyanin became colorless in the presence of carbon monoxide, and would recolor when exposed to air. Although they could see no difference between the action of carbon monoxide and an inert gas, they did not go so far as to conclude that there was no combination. Instead they stated that, if combination took place, the compound must be less stable than oxyhemocyanin, and that there was a need of quantitative data on the subject.

The data to be presented here indicate that carbon monoxide does combine with hemocyanin. Furthermore, they show that the combination contrasts sharply with that which takes place between carbon monoxide and hemoglobin, in that the carbon monoxide has a weaker, rather than a stronger, affinity for hemocyanin than has oxygen.

Methods

Limulus serum preserved with a small amount of toluene was employed as a source of hemocyanin throughout the series of experiments. The serum was equilibrated in the Barcroft type of tonometer at a temperature of 25°. The gas phase in the tonometer was analyzed by means of the Haldane apparatus, to

* Contribution No. 38.

which was attached a combustion chamber for the determination of carbon monoxide. The gas tensions were calculated by means of the equation of Bock, Field, and Adair (1924). The gases in the serum were measured by means of the Van Slyke constant volume apparatus to which was attached the special extraction chamber described by Redfield, Coolidge, and Hurd (1926). The gases were liberated from the serum in a manner described by Redfield, Coolidge, and Montgomery (1928), 2 cc. of 0.5 N KCN being used for 5 cc. of serum. Carbon dioxide was absorbed with 1 N sodium hydroxide, oxygen with sodium hydrosulfite, and carbon monoxide with ammoniacal cuprous chloride solution. In certain cases, on a duplicate determination, the carbon monoxide was not absorbed but was measured with the residual nitrogen, and its volume calculated by deducting the dissolved nitrogen. The pH of the serum was measured by means of the glass electrode.

Solubility Coefficients of Oxygen and Carbon Monoxide

The oxygen solubility coefficient has been calculated from the data of Fox (1909) for sea water, and of Redfield, Coolidge, and Montgomery (1928) for *Limulus* serum. The latter authors report a figure for the serum that is about 8 per cent lower than that for sea water at the same temperature. On the basis of this information and Fox's data for sea water at 25° and 18 gm. of chlorine per liter, a solubility coefficient of 0.022 cc. was adopted for oxygen at 25°.

In arriving at a suitable figure for the solubility coefficient of carbon monoxide it was assumed that the same relations between carbon monoxide and oxygen solubility would hold in serum as hold in pure water. According to Winkler (1901) carbon monoxide at 25° is 75.3 per cent as soluble as oxygen in water. On this basis the solubility coefficient for carbon monoxide in *Limulus* serum would be approximately 0.0166 cc. at 25°. In order to ascertain whether this value approximates the amount of gas in solution that can be measured with the technique employed, and thus afford a control for the main experiments, measurements were made on the amount of carbon monoxide taken up by sea water equilibrated with approximately an atmosphere of the gas. As a further check, the observation of Kobert (1903) that KCN prevents hemocyanin from combining with oxygen was made use of.

It seemed not unlikely that KCN might also prevent hemocyanin from combining with carbon monoxide. Consequently 1.7 cc. of a 10 per cent KCN solution were added to 30 cc. of serum. Samples of the resulting solution were then equilibrated with carbon monoxide and the gas in the serum measured. Data for both sea water and KCN-serum are recorded in Table I. It can be seen from these data that the use of 0.0166 cc. as a solubility coefficient introduces no great error, since it approximates actual measurements. The value for the KCN-serum, though slightly higher than that for sea water, indicates that serum treated with KCN does not combine with carbon monoxide.

TABLE I

Carbon Monoxide Dissolved in Sea Water and KCN-Serum at 25°

Sample	P_{CO}	CO content*		Solubility coefficient†
		(*a*)	(*b*)	
	mm. Hg	*vol. per cent*	*vol. per cent*	*cc. per cc.*
Sea water	750	1.64	1.77	0.0166
	759	1.68	1.76	0.0168
KCN-serum	710	1.60	1.78	0.0171
	712	1.61	1.80	0.0172

* In column (*a*) the values were obtained by absorption with cuprous chloride. In column (*b*) they were obtained by measurement of residual gas and by deducting dissolved nitrogen. See text.

† The solubility coefficients are calculated on the basis of the figures in column (*a*) only.

Carbon Monoxide-Combining Power of Hemocyanin

Undialyzed serum was equilibrated with approximately an atmosphere of carbon monoxide, and with lower gas tensions. The gas estimated to be dissolved was then subtracted from the total gas found in the serum. Table II contains the results obtained. In column (*a*) the figures represent the carbon monoxide values obtained by absorption with cuprous chloride. There was some question as to whether all the gas was being absorbed on account of the fact that there seemed to be too much residual gas left. In order to obtain data by a means other than absorption, as a check on the method, in certain cases the volume of all the gas other than carbon dioxide and oxygen was measured and the carbon monoxide

calculated by deducting dissolved nitrogen. The data obtained by this method are shown in columns (*b*) of Table II. This procedure was nearly always accompanied by a determination by absorption. There is a peculiar discrepancy between the figures obtained by the two methods that seems greater than could be accounted for on the basis of incomplete absorption. What is the cause of the discrepancy was not ascertained. However, it does not invalidate the conclusions one can draw from the experi-

TABLE II

Combination of CO with Hemocyanin at 25°

The sample of *Limulus* serum used had an O_2 capacity of 1.17 volumes per cent.

P_{CO}	P_{O_2}	CO content*		CO combined*		O_2 content	O_2 combined
		(*a*)	(*b*)	(*a*)	(*b*)		
mm. Hg	*mm. Hg*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*
727	16.0	2.39		0.80		0.42	0.37
743		2.30		0.68		0.30	
758	6.0	2.52		0.96		0.11	0.09
(700)†	1.8		2.75		1.22	0.11	0.11
(700)	7.3	2.55		1.02		0.12	0.10
(700)	2.4	2.59		1.06		0.12	0.12
696	8.0	2.35	2.70	0.83	1.18	0.08	0.06
724	11.6	2.43	2.67	0.85	1.09	0.15	0.12
178	5.0	0.85		0.46		0.26	0.25
164		0.75		0.39		0.10	

* See asterisk foot-note to Table I for explanation of columns (*a*) and (*b*).

† Not obtained by analysis; tonometers filled with approximately this amount of gas.

ments. The data clearly indicate a significant amount of carbon monoxide in the serum above that dissolved. If we compare them with the figures obtained with sea water and KCN-serum, the difference is very evident.

As a further test some of the serum was concentrated by dialysis so that the oxygen capacity was increased to a value about 4 times greater than the original. A sample of this serum equilibrated with about 700 mm. of carbon monoxide and a small amount of

oxygen gave a value for combined carbon monoxide of 3.3 volumes per cent and for oxygen of 0.94 volume per cent.

It will be noted in Table II that a small amount of oxygen in the presence of a large amount of carbon monoxide is still quite effective in combining with hemocyanin. This observation made it seem worth while to compare an oxygen dissociation curve established in the presence of carbon monoxide with one obtained in the

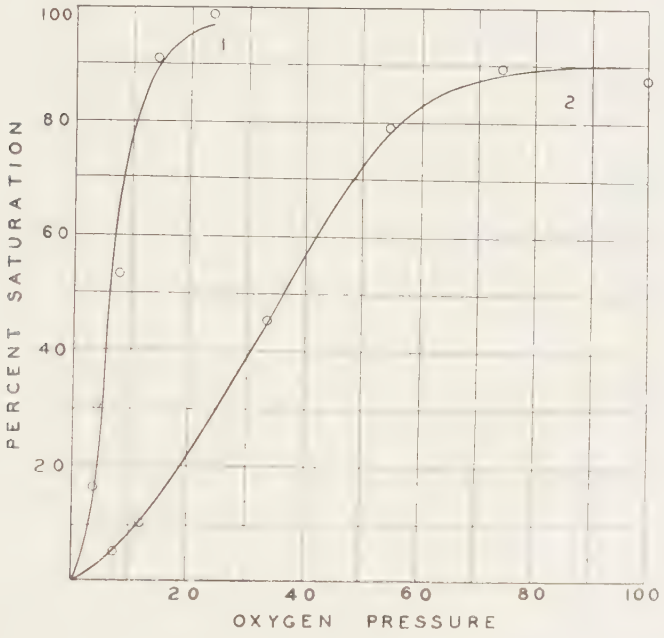

Fig. 1. Oxygen dissociation of *Limulus* serum. Curve 1 was established in the total absence of carbon monoxide. Curve 2 was established in the presence of about 700 mm. of carbon monoxide. The pH of the serum was 7.5 in both cases.

total absence of the gas. If the two curves obtained under such conditions are not superimposed, but the one in the presence of carbon monoxide is displaced to the right, it will not only be clear that carbon monoxide is replacing some of the oxygen in its combination with hemocyanin, but one will also be able to calculate the combining ratios of the two gases. The data for this study are summarized in Fig. 1. They clearly confirm the fact that carbon

monoxide combines with hemocyanin. Furthermore, the data for the dissociation curve in the presence of carbon monoxide indicate that there is sufficient carbon monoxide present to saturate the hemocyanin when there is little oxygen present. Therefore, the combining ratios of the two gases can be calculated by reading from the curve the respective pressures of the two gases at a point where each is combined with just half of the hemocyanin. It will be noted that this condition obtains at 35 mm. pressure for oxygen and about 700 mm. pressure for carbon monoxide. The combining ratios are, therefore, as 1:20; *i.e.*, oxygen has an affinity for hemocyanin that is about 20 times greater than that of carbon monoxide. This is surprising when one considers the relative affinities of these two gases for mammalian hemoglobin.

It would appear from the data presented that in carboxyhemocyanin the same stoichiometrical relation exists as does in the case of oxyhemocyanin; namely, 1 molecule of the gas is bound for each 2 atoms of copper present in the hemocyanin.

SUMMARY

Carbon monoxide combines with hemocyanin. The compound formed is less stable than oxyhemocyanin, the affinity of the gas for *Limulus* hemocyanin being only about one-twentieth the oxygen affinity. The same stoichiometrical relation exists in the compound as does in the case of oxyhemocyanin; namely, 1 molecule of the gas for each 2 atoms of copper.

I wish to thank Professor A. C. Redfield at whose suggestion this investigation was undertaken. I wish, also, to thank Mr. Walter Buck for technical assistance during the early part of the work.

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THE DISTRIBUTION AND CONDITIONS OF EXISTENCE OF BACTERIA IN THE SEA[1]

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Bacterial Population in the Sea

The two major processes with which the bacteria in the sea are chiefly concerned are the decomposition of plant and animal residues and the transformation of certain simple elements and compounds. These processes result in the mineralization of the complex organic substances and in the liberation of the elements which are essential for the nutrition of the marine diatoms and algae in an available form. But in addition to the processes of decomposition and transformation, the marine bacteria can also serve as food for marine animals, hence their synthetic activities may be of considerable importance. Our knowledge of the extent of bacterial synthesis in the sea is still very limited; although it has been suggested (7) that the bacteria in the sea are capable of developing at the expense of the dissolved organic matter, Krogh (8) obtained evidence to show that only a small part of this organic matter is available for bacterial growth. Our knowledge of the bacteria capable of causing diseases of plants and animals in the sea is also limited. One must emphasize, therefore, for the present only the first two processes, in which the bacteria were shown to take an active, if not a predominant part, and the importance of which in the cycle of life in the sea cannot be overemphasized.

Morphologically, the marine bacteria belong to the rod-shaped forms, cocci and spirilli, the last group comprising both coma-shaped and true spiral-forming organisms. Although the majority of the bacteria so far isolated from sea water are aerobic in nature, many anaerobic forms are also found to occur in the sea, especially in the bottom material. Physiologically, the marine bacteria can be divided into autotrophic and heterotrophic forms. The first group comprises those bacteria which are capable of synthesizing their cell substance out of chemical elements, or simple inorganic compounds, utilizing the energy liberated by the oxidation processes; however, these bacteria occupy in the sea a secondary place, both in abundance and in the variety of activities, although they are chiefly responsible for certain important processes, such as nitrification and sulfur oxidation. The heterotrophic bacteria are more important both numerically and due to the greater variety of their activities in the sea; they are usually found in close association with the organic substances in the sea, comprising both the residues

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of the dead plants and animals and the living forms, as well as with the organic matter in the sea bottom, namely the marine humus.

One may consider the sea as consisting of three centers or loci of bacterial life: 1. The marine plankton, with unequal distribution of the bacteria within the animal and plant plankton. 2. The marine bottom, with a marked difference in the abundance of bacteria in the sand and mud bottoms. 3. The sea water itself, which represents, however, only a poor medium for bacterial development, as compared with the other two loci; whether this is due to the limited availability of the dissolved organic matter in the sea water or to some other factor, still remains to be determined.

METHODS OF STUDYING MARINE BACTERIA

In considering the distribution of bacteria in any natural substrate, it is essential to discuss first the methods used in measuring the abundance of these organisms. Just as in the study of the bacteria in the soil, three distinct methods are made use of in the study of marine bacteria: 1. The plate culture method, whereby the material itself or a definite suspension of it in water is mixed with a favorable gelatin or agar medium and the colonies formed by the bacteria on this medium, after a certain period of incubation, counted. 2. The direct microscopic method: the bacteria in sea water must first be concentrated by filtration or by centrifuging; however, in the case of the plankton or bottom material this is not necessary, since the number of bacteria is high enough and they can be counted directly. 3. The elective culture method, largely used for demonstrating the occurrence and abundance of certain specific groups of bacteria in the sea. Another method was recently introduced, which is a combination of the last two; it consists in suspending glass slides in sea water or burying them in the bottom material; the bacteria will accumulate on the slide; they are then stained and examined. These different methods have each certain distinct advantages as well as limitations. A proper combination of these methods will result in a fair representative picture of the bacterial population of the sea.

DISTRIBUTION OF BACTERIA IN SEA WATER

The numbers of bacteria found in sea water, as determined by the plate method, are ordinarily reported to range from very few cells to hundreds of thousands of cells in 1 cubic centimeter of water. The lower figure is usually found in the case of water taken at some distance from shore, while the higher figures are reported for water taken near shore, especially at the mouth of drainage canals, sewage outlets, etc. The distance from shore, the depth of water, the nature of the bottom material, and various other factors, have been shown to exert a decided influence upon the numbers of bacteria. Russell (13), for example, has shown that the water in the Bay of Naples con-

tained 5 to 120 bacteria per cubic centimeter; the bacteria were evenly distributed at the various depths of water. Fisher (5) found the bacterial numbers to range from 0 to 29,400 bacterial cells in 1 cc. of water. The numbers were highest at the edges of ocean streams, where they came in contact with other masses of water differing in physical nature; these high numbers were believed to be due either, 1. to masses of plankton organisms which tend to die off at such edges of water streams, or 2. to a vertical mixing of the waters at the contact of the two currents, which result in the carrying of the bacteria from the lower depths upwards. Many of the samples, especially those containing the larger numbers of bacteria, could hardly be considered as representing typical water samples, since they were taken either too close to shore or were found to contain abundant algal material.

According to Drew (4), water near Andros Island contained about 15,000 bacterial cells per 1 cubic centimeter, to a depth of 500 to 700 meters, while below that layer only about 20 bacteria were found in the same quantity of water; this marked difference was explained by the fact that the upper layer of water originated locally, while the lower levels came from the deep ocean. In a recent study of the abundance of bacteria in the Clyde Sea area, Lloyd (10) found that the surface water was richest in bacteria, the numbers diminishing with depth, then increasing again at the bottom; at the surface, the numbers of bacteria fluctuated markedly, without any noticeable seasonal trend, while below the surface the numbers were more or less constant; the bacterial numbers fluctuated widely throughout the day in the surface layers, but the fluctuation was only limited at the lower depths of water.

The larger numbers of bacteria usually observed along the shore is no doubt due to the higher organic matter content, as a result of the richer plant and animal life near shore; it is not due, however, to any great extent, to the washing in of bacteria from land. Reuszer (12) reported that sea water in shallow regions, where the water tends to be mixed from top to bottom, contains much larger numbers of bacteria. This can be correlated with the greater plankton development and the greater amount of organic matter made available to the bacteria.

DISTRIBUTION OF BACTERIA IN THE SEA BOTTOM

The numbers of bacteria in the marine bottom exceed very considerably those present in the water itself. Russell (14) found that mud bottoms, at depths of water less than 200 meters, contained 70,000 to 285,000 bacteria in 1 cubic centimeter of mud, the numbers decreasing with distance from land. At greater depths of water, the numbers diminished, namely to 12,000—27,000 in 1 cubic centimeter of mud, these numbers then remaining more or less constant. The mud in the neighborhood of Woods Hole contained fewer bacteria than that of the Bay of Naples, which was correlated with

the lower temperature of the former. Most of the bacteria present in the mud were shown to be characteristic of this particular habitat. Drew (4) found in the mud off Andros Island as many as 160,000,000 bacteria per 1 cubic centimeter. Similar results were obtained by Smith (15), Lipman (9) and Bavendamm (1). Lloyd (11) observed for the Clyde Sea area a certain bacterial zonation in the mud correlated with the mud stratification; the surface layer of mud contained up to 300,000 bacteria in 1 gram of dry mud, the numbers diminishing with depth. According to Reuszer (12), the horizontal distribution of bacteria in the extreme surface layer of mud is independent of the amount of organic matter present in the mud but is largely dependent upon the degree of its decomposition; below the surface layers of mud, the distribution of bacteria is directly correlated with the organic matter content. The bacterial numbers on the continental shelf were found to decrease regularly with distance from land; however, in the deeper layers of mud, the numbers remained constant.

Samples of bottom material from true oceanic formations were found by various investigators to contain fewer bacteria; in some cases these organisms were entirely absent, at least as shown by the tests employed.

Bacteria in the Marine Plankton

In addition to the water and marine bottom, there is a third center of bacterial activities in the sea, namely, the plankton, especially the phytoplankton. Waksman and associates (16) have shown that while the water of one of the stations taken in the Gulf of Maine contained only 4 bacterial cells per 1 cubic centimeter of the surface layer of water, the zooplankton collected at the same station contained 890 bacteria per cubic centimeter of tow. A corresponding difference was found between the bacteria in the water and the diatom plankton in the water of George's Bank; on the average of two stations studied, the water contained 49 bacteria per 1 cubic centimeter and the plankton 37,000 cells in 1 cc. of tow. A study of the development of diatoms and bacteria in closed bottles carried out in coöperation with Professor Gran, brought out the fact that a definite parallelism existed between the development of the two groups of organisms; however, when the diatoms began to die off, the bacterial numbers still remained high.

In those investigations, where organic matter determinations were made, a definite parallelism was obtained between the abundance of bacteria and organic matter content; this points definitely to the fact that the most important controlling factor in the distribution of bacteria in the sea, both in the water and in the bottom, is the abundance and nature of the organic matter. Factors influencing the changes in the distribution of the organic matter will also influence changes in bacterial development.

MICROSCOPIC METHOD FOR STUDYING DISTRIBUTION OF BACTERIA IN THE SEA

The results of the investigations of the abundance of bacteria in the sea reported previously were all obtained by the use of the plate culture method. When the direct microscopic method was introduced, it was found that the actual numbers of bacteria in the sea (living and dead) are at least about a thousand times higher than the numbers obtained by the plate method, as shown by Cholodny (3), Butkewitch (2), and others. A somewhat narrower ratio, namely one of about 200:1, was obtained at the Woods Hole Laboratory, as shown by the following summary:

ABUNDANCE OF BACTERIA IN SEVERAL SAMPLES OF DIATOM TOW, AS SHOWN BY THE PLATE AND DIRECT MICROSCOPIC METHODS[1]

*Station No.**	*Nature of Tow*	*Numbers of bacteria in 1 cc. of tow*	
		Plate method	*Direct microscopic method*
1695	Surface tow	1,600,000	346,900,000
1697	Surface tow	3,500,000	202,400,000
1698	Deep tow	1,200,000	205,200,000
1699	Surface tow	600,000	234,000,000
1700	Surface tow	120,000	265,950,000
Average		1,404,000	250,890,000

These results are sufficient to emphasize the fact that the numbers of bacteria obtained by the plate method represent only a small fraction of the total bacterial population of the sea. This is due to several causes: 1. Many types of bacteria are unable to develop on the artificial medium, since no one medium can be devised which would be favorable for the growth of all bacteria; 2. Many of the bacteria in the sea, especially in the plankton and bottom material, occur not as individual cells, but as clumps which are not readily broken up in the preparation of the dilution for plating; 3. Many of the bacteria are dead, while others represent weakened cells which, for one reason or another, do not develop on the artificial media.

With all these limitations, however, the plate method offers a very convenient method for comparative studies of bacterial distribution in the sea; it should, however, whenever possible, be supplemented by the direct microscopic method.

NATURE OF BACTERIAL POPULATION IN THE SEA

As to the specific nature of the bacterial population of the sea water and the sea bottom, even a few general observations will bring out the fact that a marked difference exists between the two. In the case of the bacteria in the water, aerobic and non-spore forming organisms are found to predominate. In the bottom material, spore-forming and anaerobic bacteria are very abundant and may at times even predominate; these bacteria increase in

* Gulf of Maine or George's Bank.

[1] The microscopic counts were made by Dr. M. Hotchkiss, while in making the plate counts, the writer was assisted by Dr. C. Carey.

relative numbers with an increase in depth of the bottom material, especially in the case of mud bottoms.

The sea bottom represents a specific habitat for the development of such important groups of organisms as the nitrifying bacteria, the anaerobic nitrogen-fixing bacteria and the humus-decomposing organisms, although the available information concerning the last group is still very limited. On the other hand, the sea water, including the plankton, represents a more favorable habitat for the development of bacteria decomposing fresh organic matter, namely, the cellulose-decomposing, alginic acid decomposing, agar-liquefying, protein-decomposing and other groups of bacteria. The development of these specific bacteria in the sea will be directly correlated with the chemical nature of the plant and animal residues in the sea and their transformation products (18).

Conditions Responsible for Bacterial Distribution in the Sea

Two conditions are primarily responsible for the development of bacteria in the sea:

1. The supply of available nutrients. In addition to the abundance of organic matter in the sea water and the sea bottom referred to above, one must also consider, (a) the nature of this organic matter, (b) the extent of its decomposition, (c) presence of mineral elements essential for microbial development, and (d) abundance of elements and compounds which can be used as direct sources of energy by certain specific bacteria, such as H_2S, S, H_2, NH_3, NO_2 and CH_4. It was shown (17), for example, that in the decomposition of *Fucus* in the sea by bacteria, there is a direct parallelism between the numbers of bacteria and extent of decomposition of the algal material. The addition of a small amount of available nitrogen which favored this decomposition also favored bacterial multiplication.

2. The environmental conditions. The importance of aeration, temperature, salinity and reaction upon bacterial development is well established. When a sample of water is brought into the laboratory and placed under favorable temperature conditions, rapid bacterial multiplication takes place (6); this tends to show that the particular water contains organic substances, either in solution or in suspension, which can be used as nutrients by the bacteria, as soon as temperature conditions are made favorable.

The distribution of bacteria in the sea is thus found to be controlled by a number of factors, which are of primary importance in the cycle of life in the sea. In their turn, the marine bacteria contribute an important share to the plant and animal life in the sea, through their activities in the processes of mineralization of organic residues, and in the transformation of various elements and compounds.

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A5. 5—THE ROLE OF COPEPODS IN THE ECONOMY OF THE SEA[1]

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The fertility of the sea has long been recognized as a problem dependent upon a complex of interacting factors. Only recently, however, has investigation of the causal relationships involved been undertaken in any precise way. The subject is inseparably linked with the study of the cycle of all materials in ocean waters. In the sea, just as on land, the materials circulate in part as components of the bodies of plants and animals. Although many other relationships exist among marine organisms, the fundamental element of interdependence, as far as the production of useful forms is concerned, is one of nutrition. This relationship is often spoken of as the food chain of the sea. It would be more accurate, perhaps, to use the term in the plural since we know that nutritive material circulates among the various groups of organisms by many different channels. In the open ocean, however, one main food chain may be recognized in the general dependence of the larger animals upon the zoöplankton, and the dependence of the latter upon the phytoplankton. Here, as elsewhere, the bacteria complete the cycle, and we see again the necessity for viewing the picture as a whole in order to understand this type of problem.

Let us examine the copepods as one important link in this main food chain. In doing so we may inquire to what extent copepods illustrate the interdependence of plants and animals in the sea. The problem is obviously composed of two parts: what organisms feed on copepods and of what the food of copepods consists.

A large number of adult animals are now known to feed chiefly on copepods, notably: certain species of whales and sharks, among the large animals; the herring, mackerel, pilchard, alewife, blueback, shad, and polock, among commercial fishes; and certain Crustacea, Sagittae, and coelenterates, among the planktonic invertebrates. Many more types depend upon copepod food during various of their larval stages, and still others depend upon copepods indirectly as food for their prey. It may be concluded, therefor, that these small Crustacea are a very important element in the nutrition of larger animals in the sea.

Investigations carried out in recent years have shown fundamental quantitative relationships to exist in certain cases between the copepods and the organisms which prey upon them. Let us look for a moment at one or two examples, which are typical of most associations of this type. Hjort and Ruud have found that the Sei-whale (*B. borealis*) feeds almost exclusively on *Calanus finmarchicus*. A great and rapid development of this copepod off the coast of Möre, Norway, precedes, by a short time,

[1]Contribution no. 40 from the Woods Hole Oceanographic Institution, Woods Hole, Mass., U.S.A.

the appearance of the Sei-whale in these waters. The curve of copepod abundance during 1926 was followed very closely by the curve showing the number of these whales caught, and a distinct parallelism in the fluctuation of copepods and whales was observed to persist during the years 1926-8.

Extensive investigations of the fisheries off the east coast of England, undertaken by Hardy and by Savage, revealed the fact that the herring feed largely on the copepods *Calanus* and *Temora*—these animals sometimes comprising more than 75 per cent. of the contents of the stomach. A good correspondence was found to exist between the abundance of planktonic food in the surrounding waters and the quantity of herring caught during the years of the investigation. As an illustration of the amount of food material involved in this relationship, Savage has estimated that 300 tons of plankton would be required for one meal for the 1,000 million herring landed at English east coast ports during 1926.

Food not only affects the numerical size of the catch but also regulates to a great extent the rate of development and the quality of the adult fish. Savage has shown that when the food supply is poor, maturity is retarded, and it is suggested that in these circumstances, herring which should spawn in winter may not do so until the following spring. Wimpenny has found that herring feed greedily upon copepods just before they spawn, and that this occurs at a time of year when the fat content of the plankton is increasing. "It seems", he concludes, "that the great congregation of East Coast herring owes its successful reproduction and the growth capital for its eggs and young to the presence of rich zoöplankton shoals in the North Sea." The type of plankton consumed similarly affects the condition of the pilchard off the coast of British Columbia, for Hart and Wailes have shown that when this fish has eaten "green feed"—consisting of diatoms—the catch possesses a low oil content, whereas red feed—consisting of copepods—is accompanied by a high oil yield.

These quantitative and qualitative relationships between the plankton and the commercial fisheries induced Hardy to develop his "Plankton Indicator". The green, red, or white colour of the straining disk of this instrument reveals the nature of the plankton and thus enables the fishermen to test the fishing with a certain degree of accuracy before they cast their nets. As more and more is learned about the dependence of such fish as mackerel and herring upon the plankton, the possibility is increased that this type of device may be used successfully on a large scale.

Copepods may exert an important influence on the fisheries in still another way, namely, in causing or modifying the migrations of fish. Wimpenny found that during the summer of 1926 there was some indication that the maximum fat content of the zoöplankton moved further and further south in the North Sea. This was coincident with the southward movement of the herring fishery. The vertical distribution of the fish may be affected by the level of abundance of the plankton. In July, 1922, Savage found that the herring drifters in the Shields fishing area, using nets which would scarcely touch ten fathoms, were not taking many fish and that the planktonic food forms were absent down to this level. The well-known diurnal vertical migrations of copepods undoubtedly affect the level of the fish that subsist upon them, directly or indirectly,

and consequently influence the relative success of the day or night fishing at the surface or at the bottom.

It is important to inquire to what extent pelagic invertebrates deplete the valuable copepod population. We are concerned also because certain species which feed on copepods destroy the eggs and larvae of commercial fish as well. On the other hand, some forms, such as *Euthemisto,* may themselves be important as food for fishes. Bigelow reports that the stomachs of *Euthemisto* taken in the Gulf of Maine are often crammed with copepods. Decapod larvae and Sagittae are also known to feed on these animals. But Bigelow believes that "of all the members of the plankton the most destructive to smaller or weaker animals are the several coelenterates"—particularly the ctenophores *Pleurobrachia* and *Beroë.* The destructive nature of *Pleurobrachia* was confirmed by observations in Monterey Bay, for in these waters copepods were found to be relatively scarce wherever this genus was notably plentiful.

Since the importance of copepods as a source of food is recognized in these various ways, let us proceed to investigate the food supply of these little pelagians. The ultimate cause of a fluctuation in the abundance of a species of fish may be due, not to a disturbance in their immediate food supply, but to a change in a remote link of the food chain. Thus Allen demonstrated the existence of a relationship between the number of hours of sunshine in February and March and the number of mackerel landed by the English western fishing fleet in May during the years 1902-8. Bullen had previously shown a correspondence between the abundance of zoöplankton and the abundance of mackerel in this region. Since copepods were believed to subsist upon diatoms and peridinians, at least indirectly, and these were known to be vitally dependent upon light, Allen suggested that the relationship observed between the mackerel and the sunlight was brought about through these intermediate elements in the food chain.

A general dependence of copepods upon phytoplankton of this sort has been recognized now for a long time, but the specific food organisms are still poorly known. In those parts of the sea where extensive observations on the seasonal changes of the plankton have been carried out, increased production of copepods is almost invariably reported as following a burst of diatom growth. But to conclude from this that copepods live on diatoms is a *post hoc propter hoc* argument, and we are permitted to inquire whether there is a true causal relationship here. Bigelow found that an unmistakable *decrease* in the amount of zoöplankton present in the Gulf of Maine took place coincidently with the local flowering of diatoms. If copepods really feed on diatoms, it is difficult to understand why this diminution of numbers should have occurred. Johnstone has reported the same phenomenon from the other side of the Atlantic and makes the general statement that whenever diatoms and peridineans are abundant, the microcrustacea are scarce. Conclusions drawn on food relationships from isolated observations may be entirely erroneous. For example, one cannot say whether the absence of diatoms in a body of water where copepods abound means that the latter do not eat these plants and are, therefore, not attracted to—or even avoid—zones of their abundance, or whether it means that the observation occurred at a moment when the copepods had just finished a diatom meal—denuding the water in the

process. If diatoms are indeed the chief food of copepods, the two organisms ought sometimes to be found together, the copepods showing signs of rapid growth and reproduction.

In an effort to get to the bottom of this matter, the alimentary canals of hundreds of copepods were subjected to microscopic examination by Dakin, by Esterly, and by Lebour. A major part of the recognizable material was found to be composed of diatom fragments, but peridineans, silicoflagellates, coccolithophorids, and other protista were present in varying degrees of abundance. In some cases remnants of other copepods occurred in the intestines. It was realized from the beginning that only such objects as are provided with calcareous, silicious, or chitinous parts of characteristic outline would be discovered by this type of examination. Almost always the guts contained more or less of a green substance which apparently represented the remains of shell-less unicellular plants. Doubt thus remained as to the extent to which copepods might feed on tiny, naked organisms which would be quickly digested, leaving no traces at all. A strong suspicion that such material might be an important element in the copepod diet was raised by the surprisingly large number of cases in which the guts were found to be empty.

Another possibility for the nutrition of copepods appeared with Pütter's theory that aquatic animals have the power to absorb dissolved organic substances directly from the surrounding water. Many investigators feel that the very presence of highly specialized feeding mechanisms itself proves the dependence of the animal possessing them upon particulate food. Johnstone, however, claims that such mechanisms could perfectly well have developed as an extension of the power of direct absorption just as in the case of insectivorous plants. Copepods appear to possess a true filtering apparatus which, according to Naumann, is so efficient in *Diaptomus* that particles less than one micron are retained. But the average abundance of suspended food in the water is so low that an absurd amount of water would have to be strained by any filter feeder. It is entirely reasonable to assume, however, that the food particles occur in patches, and that the feeding animals do not swim aimlessly about with their mouths open, but make use of their sensory equipment to locate zones of abundance and to remain near them. Krogh, after a survey of all the evidence, comes to the conclusion that there can be very little doubt that the majority of aquatic animals are able to obtain sufficient food in the ordinary way.

The conflicting nature of the evidence and opinions just discussed shows the urgent need for critical experimentation on this whole subject of nutrition. Yonge points out that,

> Nothing can be based on the sparseness of intestinal contents in any animal. Carnivores—coelenterates to fishes—digest with remarkable speed, while the flagellates and peridinians secured by the ciliary feeding mechanism of molluscs or the setous mechanism of the lower crustacea are unrecognizable before they have even reached the stomach.

The material which is actually found in the alimentary canal is not necessarily food—it may be indigestible material, unavoidably taken in with the food, and destined to be ejected unused. For this reason Yonge asserts that the digestive enzymes present in each animal should be studied.

The few experiments on the nutrition of copepods which have actually been carried out represent progress in the right direction and it is

hoped that this work can be extended in the near future. Pütter's theory has recently been tested for these animals by Bond with negative results. In a few cases copepods have been kept alive in the laboratory for short periods of time on cultures of diatoms (notably *Calanus* on *Nitzschia* by Crawshay). We have practically no evidence on the digestive enzymes of copepods, however. Our most pertinent information is that derived from Van Dehn's feeding experiments with *Daphnia* in which he showed that protein, fat, and starch can be digested, but not cellulose or pectin.

From the foregoing discussion of the main food chain involving marine organisms, we may conclude that the role of copepods in the economy of the sea is that of middle-man. Very instructive diagrams, which illustrate this situation admirably, have been prepared by Hardy and by Dakin. The former shows graphically the relation of the herring at various stages of development to all the important elements of the plankton community. The latter reveals, in a series of drawings, the dependence of the tiger flathead—the most important constituent of the trawler catches on the New South Wales coast—upon the small fish *Apogonops*. This fish seems to feed very largely upon minute Crustacea which in turn depend upon the phytoplankton. In each case the various organisms are joined by a maze of connecting links representing the fundamental interdependence of plants and animals in the sea. The central position of the copepods in both diagrams bespeaks once again the importance of these animals as the chief intermediary between the phytoplankton and the commercial fish. The study of copepods is, therefore, one subject upon which investigators of every branch of oceanography may well concentrate their efforts in an attempt to understand the factors controlling the fertility of the sea.

Contribution No. 41

FACTORS AFFECTING THE VERTICAL DISTRIBUTION OF COPEPODS

By GEORGE L. CLARKE

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For planktonic organisms the world is three dimensional; and for them the vertical dimension is perhaps the most important. The factors which bring about the vertical distribution of planktonic populations are therefore of considerable interest (*cf.* Russell, 1927). Let us investigate these factors in the environment of a copepod, since we have considerable information about this type of animal, and inquire as to the *modus operandi* of each. The principles involved are universal in their application and may be used in the interpretation of the vertical distribution of any planktonic community in any large body of water. Many apply to problems of horizontal distribution as well.

The depth at which a copepod finds itself is due in part to mechanical forces which transport it bodily through the water and in part to its own swimming activity. In response to gravity, the most important of the former, the population is continually tending to sink. Immature copepods, having larger areas for their masses, do not fall as rapidly as do the adults. For a given individual the rate of sinking depends upon the viscosity of the water which in turn is affected by the temperature and pressure. Under laboratory conditions large copepods have been observed to sink at rates of about 40 cm./min. (*cf.* Parker, 1902; Gardiner, 1933). The action of gravity is therefore a serious matter for these forms. In regions where upwelling exists on a large scale, the animals may be carried toward the surface by the current faster than they tend to sink through the water. In most parts of the sea, however, vertical movements of water masses are of limited extent and often take place in a downward as well as an upward direction.

Turbulence due to wave action is continually causing suspended objects to be dispersed throughout the water mass. Plankton concentrated near the surface will tend to be carried downwards, and populations in deep layers will tend to be transported toward the surface by the vertical component of this force. Now the coefficient of turbulent conductivity is greatest at the surface and diminishes with depth. This means that for organisms inhabiting an intermediate zone, upward scattering will take place faster than downward scattering. For the vertical distribution of the phytoplankton turbulence is obviously of great importance. But this factor is ordinarily of negligible effect for such strong swimmers as copepods according to calculations kindly furnished by Prof. C. G. Rossby. In the Gulf of Maine, for example, it is

[1] Contribution No. 41.

only during the winter storms that turbulent conductivity is sufficiently strong to have an appreciable influence upon the distribution of the copepod population to any considerable depth, and in the summer its effect would be felt only in the surface stratum during the severest storms.

Besides translocational influences of this sort the relative distribution of the population may be affected by the actual addition or removal of individuals at particular depths. Rapid reproduction within a limited zone is a case in point, and although the affect would probably not be appreciable in a copepod community, it undoubtedly is important for the phytoplankton. The distribution would be modified in the reverse manner by the consumption of individuals at certain depths by other organisms or by death resulting from movement into strata where lethal conditions exist.

Copepods are rapid swimmers for their size. From laboratory experiments we know that copepods can swim in a horizontal direction as fast as 136 cm./min. (Welsh, 1933). Such animals would theoretically be able to swim upwards at this rate less the sinking rate, or approximately 100 cm./min. If such a speed can be maintained over any considerable period of time, it would appear that the vertical distribution of copepods is chiefly determined by the swimming activity of the animals themselves. We must, therefore, proceed to inquire to what extent the hydrographic and biological features of the environment are important in controlling the direction and the speed of movement of the copepods.

It is essential to establish the fact at the outset that an organism cannot respond in a directional manner to any environmental agent unless the difference in the stimulation of two parts of its body is discernible. This means that the intensity of the agent in one direction must be perceptibly greater than in another direction. For example, in large bodies of water, the change of temperature with depth is certainly too gradual for any organism as small as a copepod to perceive in which direction it is becoming greater or in which direction becoming less. The most rapid change of temperature with depth observed during observations on copepods in the Gulf of Maine was a change of 1 degree in 1.5 meters (see Figs. 1 and 3). Even for this extreme case the difference in temperature at the two ends of an organism 5 mm. long would be only 0.003°C. Temperature may, and often does, affect vertical distribution indirectly, as is described below, but it cannot of itself orient the swimming movement of the organism.

The ever-present force of gravity may be listed as of prime importance in regulating the swimming of copepods. Gravity exerts its pull from one direction and the copepods are oriented in the vertical plane accordingly. The animals may also respond positively or negatively to this directional force by geotropic swimming movements. Whether the geotropism will be positive or negative depends upon physiological regulation by internal and external factors.

Light is a second agent which may have a directional effect on the movement of the copepod. This factor can operate only during the daytime and near the surface where the direction of propagation of the light is perceptible. This condition may obtain to considerable depths since Poole and Atkins (1929) found that even at 25 m. the vertical light is twice as great as the horizonal light. The sign of phototropic swimming is determined by the intensity of light and by other factors such as temperature. At depths where

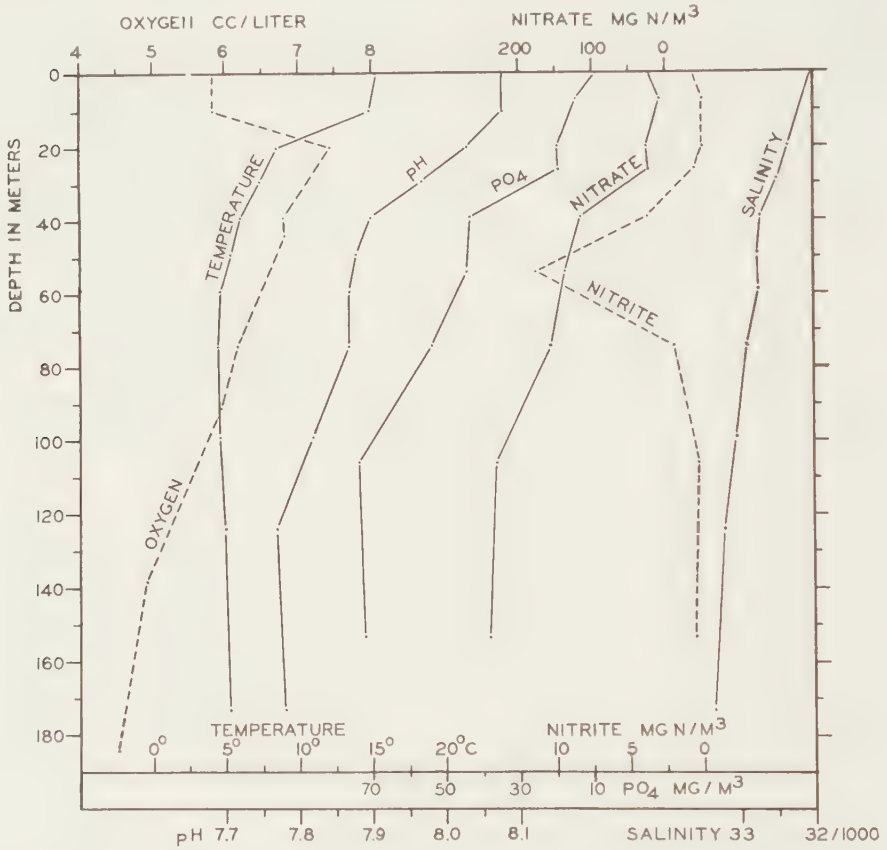

FIG. 1. Variation of hydrographic factors with depth. Station 1287, July 10-12, 1932, Lat. 43°04′ N. Long. 69°24′ W. (except for oxygen which is for Station 1285, July 9, 1932, Lat. 42°34′ N. Long. 69°37′ W.)

the illumination is completely diffuse, phototropic responses can no longer take place. The intensity of the light present may, however, affect the sign of geotropism.

Temperature, as already pointed out, changes so gradually with depth (see Fig. 1) that the difference in amount of heat reaching a copepod from any two directions is imperceptible. Under these circumstances no thermotropism can exist. Temperature can, however, affect the sign of geotrop-

ism and phototropism. Secondary affects of this sort have been demonstrated repeatedly in the laboratory (*cf.* Rose, 1929). Accordingly, temperature may play a very important rôle in controlling the vertical distribution of plankton, but its action is indirect and it can affect only those animals which exhibit geotropism or phototropism.

The vertical gradients of the salinity, the pH, the concentrations of oxygen and other substances which are widely distributed through the water are again almost certainly too gradual for the organism to perceive the change (see Fig. 1). Even if the copepod possess sense organs specialized for the detection of these substances, orientation cannot be brought about if the difference in the stimulation of two parts of the body is not above the threshold of discrimination for the organism. It is impossible for the copepod to "tell" in which direction salinity, for example, is increasing, and in which decreasing. If such features of the environment do affect the vertical movements of copepods at all, they can do so only by modifying geotropism or phototropism.

The presence of other organisms may influence the distribution of copepods. The direction in which actual contacts occur can, of course, be perceived and a copepod may even be able to tell whence came a vibration or a motion of the water. Avoiding reactions are responsible in part for the dispersion of the animals; processes of the reverse sort bring the animals together in swarms. During the breeding season male copepods have been observed in the laboratory to turn quickly and attempt to grasp other individuals which chanced to brush past them. Copepods undoubtedly do react in a directional manner to dissolved substances of a characteristic sort emanating from a highly concentrated source. A true chemotropism of this sort probably exists in the neighborhood of a relatively large decaying organism or in the wake of an individual of the opposite sex. The concentration gradient would be steep and hence perceptible in such cases as distinguished from those mentioned in the preceding paragraph.

Vertical movements in relation to a food supply have been suggested as important in the distribution of copepods (Worthington, 1931). We have evidence that copepods do not pick out discrete particles in feeding, but filter everything in their path (Cannon, 1928). If this is true, can they direct their swimming movements into regions where food is abundant? Here again the question of gradient and discrimination threshold is encountered. The change in concentration of phytoplankton organisms even at the limits of a zone of "abundance" is extremely gradual in comparison with the size of a copepod. Under these circumstances it is difficult to believe that a copepod could perceive in which direction food organisms are increasing in abundance and in which direction they are decreasing. This reasoning would apply to stimulation received both from actual contact and from diffusing chemical

substances. If the organism fed by swimming after separate particles of food, then it would be automatically carried toward zones of greater concentration by the laws of chance. For continuous filter feeders under conditions varying too gradually to be perceived, it seems necessary to conclude either that only those animals survive which happen to swim in the right direction, or that the absence of food in the digestive tract influences geotropism or phototropism suitably to bring the animals to levels where edible organisms are ordinarily found.

There are, then, only two or possibly three ways in which a copepod may tell "which way is up": orientation may be brought about by gravity, by light, or possibly by stimuli received from other individuals. However, it is not the direction of movement alone which determines the configuration of the copepod population; the speed and duration of swimming also contribute to the results. We know from many laboratory observations that the light intensity and the temperature of the medium influences profoundly the rate of movement of planktonic animals. The concentration of dissolved substances may operate in a similar fashion under certain circumstances.

When tested in the laboratory an increase in illumination or in temperature ordinarily causes an acceleration of the swimming rate, and a decrease in either results in retardation. But these characteristic effects on swimming speed appear to operate in the wrong direction for the usual situation in nature. Consider a copepod inhabiting a certain stratum of water. In the typical case the animal would appear to be confined to this range because the light intensity or the temperature is too high above the upper limit and too low below the lower limit. Now the animal must always be swimming upwards to some extent to compensate for its tendency to sink. If our laboratory observations are correct, we should expect the copepod to increase its rate of swimming when it rose into brighter and warmer water layers. This would tend to carry it still higher in the water. Conversely, whenever the animal dropped into deeper water layers where temperature and illumination are reduced, we should expect, on the same basis, that its swimming activity would diminish and that the animal would consequently sink to greater and greater depths. Undoubtedly many animals on the outskirts of the population are continually being killed off as they are carried farther and farther from tolerable conditions in response to these reactions. At the upper limit of the range the increasingly lethal conditions would probably cause paralysis before becoming actually fatal. The organism would consequently sink and if it reached favorable conditions soon enough, might thus escape death in this case. However, this mechanism would just make matters worse at the lower limit of the range: paralysis would result in even faster sinking away from the habitable zone. But copepod populations do succeed in maintaining themselves above certain definite limits. The conclusion seems

unavoidable, in this case at least, that the decrease in illumination or in temperature, although it retards swimming, also reverses the sign of geotropism (or of phototropism) with the result that the copepod is stimulated to swim upwards.

Finally the duration of the copepod's swimming movement is in part responsible for the actual distribution which we observe at any given moment. A study of the seasonal changes in vertical distribution is not concerned with this third factor because there is plenty of time for an equilibrium to be reached. However, in an investigation of the depth of the copepod population at different hours of the day, the duration of the movement is decidedly important. There may not be time enough for equilibrium to be reached before a change in environmental conditions produces new reactions in the animals. This whole question is obviously bound up with the problem of diurnal migration which has been extensively studied in recent years (Russell, 1931; Worthington, 1931; Southern and Gardiner, 1932; Nicholls, 1933; Clarke, 1933, 1934). A specific example of diurnal migration will illustrate this point and also reveal the application of the questions discussed above to a practical problem.

Figure 2 shows diagrammatically the diurnal migration of the adult females of *Metridia lucens* during July 10, 11, and 12, 1932 at a station in the Gulf of Maine (Clarke, 1933). The lines of equal light intensity (1000 μW/cm^2, 100 μW/cm^2, etc.) drawn in the figure indicate the changes in submarine irradiation to which the copepods were subjected during the course of each day. Inspection of the diagram reveals the fact that in general the zone of maximum abundance of *Metridia* occurs between the light intensities of 10 μW/cm^2 and 1 μW/cm^2 (or less) and rises and falls coincidently. Early in the morning and late in the evening the light intensity changes very rapidly, and we might expect that the animal could not "keep up" with its zone of optimum illumination. For example, it can be roughly calculated from the figure that the position of the 1-microwatt line rises about 46 meters in the hour between 19 and 20 o'clock. To keep pace with this a copepod would have to swim vertically upwards at an average speed of 77 cm/min. Such a speed would actually be possible according to the calculations made earlier. But for populations living at greater depths during the daytime and exhibiting a positive phototropism during the afternoon, the intensity of light reaching them may well become imperceptibly weak before the animals ever reach the surface strata. However, geotropism frequently is in operation also, as in this case of *Metridia*, since the copepods continued to swim toward the surface after complete darkness.

Light is obviously the chief factor which changes during each daily period but other factors which may affect vertical distribution directly or indirectly should be examined for diurnal fluctuations. Hydrographic measurements

FIG. 2. The diurnal migration of the adult females of *Metridia lucens* during the 48-hour period of Station 1287 (July 10-12, 1932, Lat. 43°04' N. Long. 69°24' W. The changes in the light intensity are indicated by the lines representing the depth at which 1000 μW/cm^2, 100μW/cm^2, etc., occurred. (One microwatt/cm^2 may be taken as approximately equal to 3 meter-candles as used by Poole and Atkins, 1929). Where the lines are broken, a shortening of the time scale is indicated merely; observations were not discontinued during these periods. The temperature curve for this station is reproduced in the lower right-hand corner.

made within a day or two of these observations on *Metridia* showed that no significant diurnal changes in the hydrogenion concentration, in the oxygen content, or in the temperature were taking place in this region. Variations in the depth of food organisms during such short periods are likewise highly unlikely. However, a year later at another station in the Gulf of Maine more complete measurements of the temperature told quite a different story. The results are shown in Fig. 3. Measurements of temperatures were made every five meters to a depth of fifty meters and the series was repeated 19 times during the 54-hour period. The isotherms show two sharp peaks: one at 5 o'clock, July 15, and the other at 6 o'clock, July 16. There are three less prominent peaks alternating with these, the whole series showing a very decided tidal rhythm. The phenomenon is obviously controlled by the tides but the complete explanation is not known.

We are accustomed to think of temperature changes in the sea as being very gradual. Such diurnal changes as are known to occur are ordinarily due to heating by the sun. This effect can be seen in the present case in the variation of the temperature at 1 meter. The difference between the highest and the lowest temperatures at this depth was only 0.9°C. But at a depth of 28 meters the temperature changed from 7°C. to 12°C. between 5 and 8 o'clock of July 15. In this extreme case there was a variation of 5° in three hours! If temperature changes of this magnitude occur generally in the Gulf of Maine or elsewhere, the vertical distribution of plankton must be profoundly affected. Hydrographers, too, must reckon with such diurnal fluctuations.

It would be impossible, of course, for a body of water actually to change

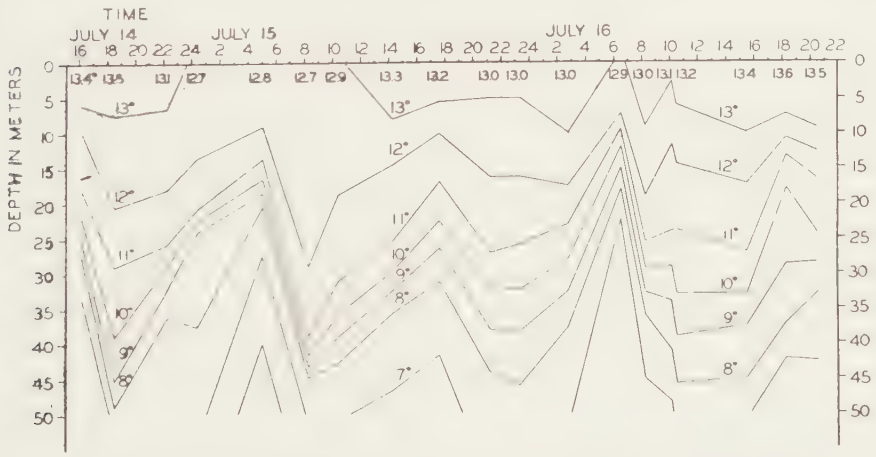

FIG. 3. Variation of temperatures down to 50 meters during a 54-hour period.

Station 1722, July 14-16, 1933, Lat. 42°50′ N. Long. 67°05′ W. Nineteen series of measurements were made at the hours indicated (24-hr. scale). The temperatures at the top are for the 1 meter level. The black dots indicate the half-degree points.

its temperature to such an extent under these circumstances. The effect must have been produced by a vertical movement of whole strata of water. For example, the position of the thermocline is seen to have been displaced downwards 25 meters during the 3 hours following 5 o'clock, July 15. Now the plankton would tend to be carried bodily up and down with the masses of water in which they are suspended. The question at once arises whether active forms like the copepods could maintain their positions in depth by swimming in the appropriate direction. This would be possible since the maximum rate of water movement is only about 14 cm/min. If they did so, they would experience wide changes in the temperature of their surroundings.

Observations on the diurnal migration of *Calanus finmarchicus* at this station (Clarke, 1934) reveal a vertical movement of the population from depths as great as 138 m. during the day to the 6 meter level at night. These animals were "on the go" continuously except during the middle of the day and the middle of the night. At these periods the population was respectively below and above the zone of extreme temperature change. But at dawn and at dusk *Calanus* carried out its migration right through the thermocline. The responses of this animal to gravity and to light are therefore stronger than those to temperature.

In conclusion let us review our findings in regard to the action of all these environmental factors upon the copepods. Of the mechanical forces, sinking due to gravity is the only one of serious importance. Gravity can be overcome by swimming, and, in the last analysis, the vertical distribution of copepods is determined by their own swimming activity. Directional movement can be caused only by gravity, light, and possibly the presence of other organisms, but other factors may have an indirect effect through modification of whatever geotropic or phototropic reactions are in operation. The speed and duration of the swimming also play their parts in controlling distribution. Speed is modified ordinarily by illumination and temperature. The time available for swimming depends upon the rate at which the environment becomes altered. Light changes more rapidly than any other factor; but temperature, in one case at least, is shown to fluctuate profoundly. Even in respect to these factors copepods can adjust their distribution with the necessary speed through their own swimming activity.

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NOTE

During the discussion which followed the presentation of this paper at the meeting of the Ecological Society of America on December 30, 1933, Dr. V. E. Shelford called my attention to certain experiments on the responses of animals to gradients performed by him and his associates.[1] In these experiments it appears that the animal "turns back from a weaker stimulus after encountering a stronger one; the threshold of stimulation is lowered." It is possible that the sensitivity of an animal in nature may be similarly increased. However, a limit must eventually be reached,—that is, a situation where the gradient of such a factor as temperature is so low that the animal, in its most sensitive condition cannot "perceive" in which direction the intensity is increasing and in which decreasing. Another sort of increase of sensitivity would result from rapid swimming in a direction perpendicular to the gradient thus augmenting the rate of intensity change in time. This might result in an "avoiding reaction" which would not otherwise have been produced. Here again a limit is reached when the gradient is so low that no response is evoked at the maximum swimming speed. Even if copepods possess some sort of "memory," this would be of no service for orientation when in a uniform environment, such as the open ocean, any more than it is for a man lost in a fog. There is, then, a definite limiting threshold gradient below which the organism cannot respond directionally. Obviously the precise value of the threshold gradient for copepods can be found only by

[1] Shelford, V. E., A comparison of the responses of animals in gradients of environmental factors with particular reference to the method of reaction of representatives of the various groups from protozoa to mammals. *Science*, N. S., 48: 225-230, 1918.

direct experiment. However, the evidence presented in the foregoing paper indicates that in the ocean the gradients of such factors as temperature are below the threshold for copepods. It does not appear that the experiments of Shelford and his associates nor those of any other investigators with which I am familiar cast doubt upon these conclusions.

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THE EFFECTS OF LAND DRAINAGE UPON THE EXCESS BASES OF SEA WATER [1]

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The total titratable alkalinity of sea water, that is, its buffer capacity for acids, tends to reach an equilibrium such that the total buffer capacity divided by the chlorinity is a constant. This fact has been established by several investigators working on samples from the North Sea and its vicinity and by Thompson and Bonnar (1931), who studied the waters of the region of the San Juan archipelago and Puget Sound. The results of Mitchell and Rakestraw (1933) on Atlantic waters of the Cape Cod and Bermuda regions also revealed the tendency toward constancy of ratio. It is to be expected that when the only important change in the sea water composition is that due to the addition or withdrawal of water by rain or evaporation, this and similar ratios should be constant.

It appears, however, that the ratio as observed in different regions is not necessarily the same. The differences might be due, in part at least, to variations in methods of measurement but this appears not to be the entire explanation (Mitchell and Rakestraw, 1933). It would seem, then, that the oceans are not in apparent equilibrium as regards the ratio of excess base to the salts. But although some approach to a constant buffer capacity-chlorinity ratio is discernible in ocean waters, such is not the case, generally speaking, in the waters of sounds, bays and inlets where equilibrium conditions are more actively disturbed.

Thompson and Bonnar have discussed factors which alter the ratio. Among them are photosynthesis and other effects of plant life and of animal life and the effect of land drainage. Our own experience has shown the latter to be a very significant factor tending, as Thompson and Bonnar found, to raise the buffer capacity-chlorinity ratio but showing, in our observations, great variability with locality, character of the soil and rapidity of drainage, that is, whether surface or deep drainage. It has even appeared possible to us that the character of the soil in different regions of the earth might vary sufficiently in potentiality for yielding bases to the sea to account in part for the differences in the ratio as apparently found in different ocean areas.

[1] Contribution No. 42 from the Woods Hole Oceanographic Institution.

A satisfactory illustration of the land drainage effect was obtained by observations on Narragansett Bay. It is a relatively long and narrow body of salt water. No large, long rivers enter it so that the drainage which it receives is of comparatively local origin. Measurements of buffer capacity were made by a method described in a previous communication (Mitchell and Rakestraw, 1933). The results of typical observations, as shown in Table I, reveal what is probably demonstrable for any similar body of water, namely, that the lower the salinity the higher, in general, is the buffer capacity-chlorinity ratio. This is a tendency rather than an exact relationship because of the disturbing

TABLE I

The Relative Buffer Capacity of Narragansett Bay Water Samples Arranged in Decreasing Order of Chlorinity

Chlorinity *grams per liter*	Ratio $\frac{BC}{Cl}$	Chlorinity *grams per liter*	Ratio $\frac{BC}{Cl}$
19.50	0.1143	15.94	0.1272
19.00	0.1153	15.93	0.1242
18.10	0.1222	15.50	0.1191
17.90	0.1227	15.50	0.1216
17.38	0.1210	15.40	0.1275
17.38	0.1217	15.22	0.1266
17.02	0.1208	15.05	0.1307
17.02	0.1208	14.96	0.1229
16.85	0.1189	14.60	0.1283
16.84	0.1171	14.52	0.1383
16.73	0.1186	14.45	0.1338
16.72	0.1239	14.06	0.1345
16.55	0.1200	13.88	0.1364
16.48	0.1223	13.15	0.1258
16.36	0.1214	13.04	0.1425
16.31	0.1251	11.92	0.1535
16.20	0.1258	5.40	0.1630
16.16	0.1237	3.61	0.1690
16.05	0.1188		

effects of tidal currents, storms, freshets and local conditions such as the relative abundance of plant and animal life.

In order to demonstrate more clearly the relationship between dilution of estuary waters and their buffer capacities, observations were made upon various series of mixtures of salt water with fresh water from selected sources. In every case progressive dilution caused a rise in the buffer capacity-chlorinity ratio such that the latter plotted as ordinates against the chlorinity as abscissas gave a smooth curve. The rise in the ratio with addition of fresh water was not, however, the same in all cases. To illustrate typical variations, a number of the curves obtained are shown in Figs. 1 and 2.

Curves *A, B* and *C* were obtained by dilution of Narragansett Bay water with that of the Pawtuxet River which drains the watershed supplying the city of Providence. The fact that observations on the undiluted salt water from the bay, giving the lowest point of each curve, do not yield closely approximating results is due to the circumstance that water collections were made at different stages of the tide and in one case (*A*) shortly after an easterly storm which raised the salinity of the water above its usual value. This is in part the explanation of the different levels of the curves. But it may also be seen that the slopes of the curves are different.

Curve *A*, obtained on March 7, 1933, illustrates the effect of an easterly storm in that the undiluted bay water has a relatively high

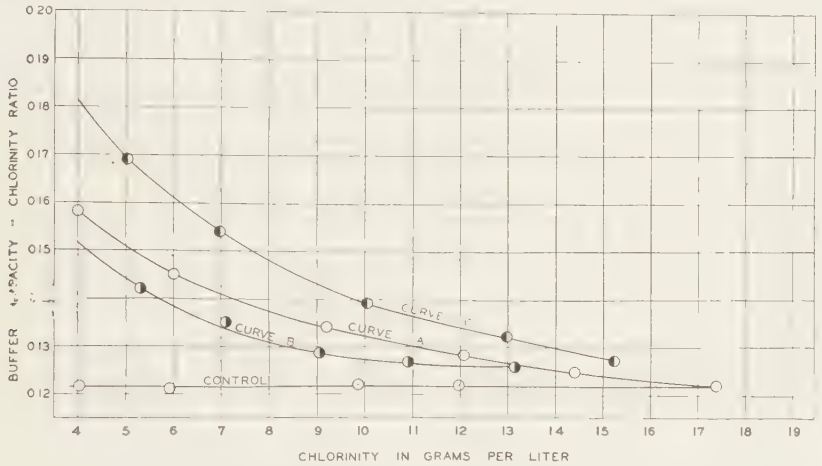

FIG. 1. Effect of successive dilutions of Narragansett Bay water with Pawtuxet River water. Curve *A*, in March, Curve *B*, after a freshet in April, Curve *C*, in June. For the control, dilutions were made with distilled water.

salinity and a low ratio. The rise in the ratio with progressive dilution is an intermediate one increasing from 0.1253 (Cl, 14) to 0.1583 (Cl, 4).

Curve *B*, obtained on April 11, 1933, illustrates the effect of mere surface drainage. There had been a light snowfall followed by especially heavy and prolonged rain. The river was at spring freshet level. The ratio increased only from 0.1252 (Cl, 14) to 0.1520 (Cl, 4).

Curve *C*, however, illustrates the more typical effect of the true soil drainage of this area. Sampled on June 13, 1933, after a period of approximately normal rainfall, the river water raised the ratio from 0.1293 (Cl, 14) to 0.1820 (Cl, 4).

There is then a seasonal variation in land drainage effects upon the

titratable base of estuary waters. The larger the proportion of deep soil drainage to surface run-off, the greater is the effect in this particular type of drainage area.

Curves *D* and *E* illustrate the relatively large effect of deep soil drainage in another way. The salt water in these cases came from Sakonnet Point at the more easterly of the two mouths of Narragansett Bay. The fresh water in the case of curve *D* was taken from a pond about one mile inland from the Sakonnet shore and in the case of curve *E* from a clear deep spring near the edge of the same pond. The ratio in curve *D* increased from 0.1240 (Cl, 16) to 0.1381 (Cl, 6) while in curve *E* the increase was much greater, from 0.1182 (Cl, 16) to 0.1490 (Cl, 6).

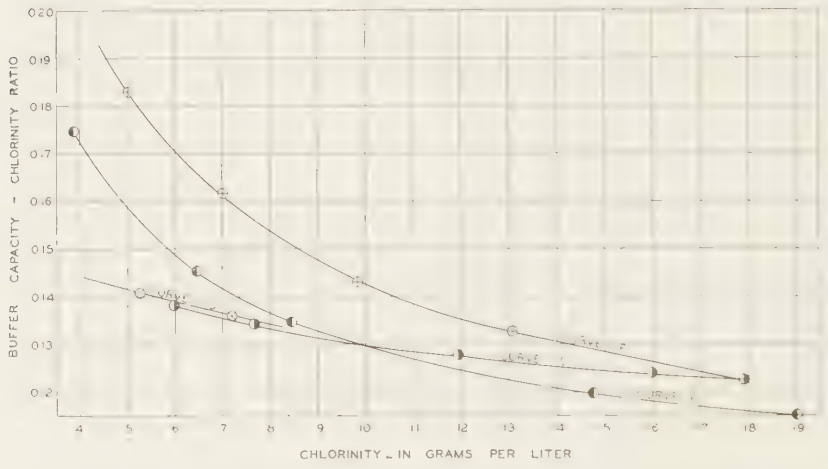

FIG. 2. Effect of successive dilutions of sea water with fresh water from various sources. Curve *D*, with pond water, Curve *E*, with spring water, Curve *F*, with pond water from swampy soil, Curve *G*, with pond water from sandy soil.

Another factor influencing the drainage effect is the character of the soil. Curves *F* and *G* illustrate this fact. The sea water used for both of these sets of measurements was taken from Woods Hole Harbor on June 14, 1933, after a period of only moderate rainfall. The fresh water used for the dilutions of curve *F* was collected on the same day from a nearby pond in a swampy area, while that used for curve *G* came from another pond about four miles away entirely surrounded by very sandy soil. Curve *F* shows a rise of the ratio to 0.1832 (Cl, 5) but curve *G* rises only to a ratio of 0.1416 at the same dilution. Apparently a barren, sandy soil yields comparatively little excess base to its drainage water.

The curve at the bottom of Fig. 1 indicates data for a control upon the accuracy of the measurements and the reproducibility of the results. It was obtained by adding distilled water to a sample of Narragansett Bay water. It shows no change, in excess of the limits of observational error, in the ratio even when the chlorinity is diluted to 4 grams per liter. The probable error of a single determination of the ratio is found to be approximately $\pm$ 0.0007, a value in accord with numerous other estimations of the probable error of this method.

Summary

1. Land drainage tends, in general, to raise the buffer capacity-chlorinity ratio of sea water.

2. Heavy rains measurably decrease the proportional effect.

3. The effects of deep soil drainage are greater in raising the ratio than are those of mere surface drainage.

4. The character of the soil alters the effect. Sandy soil may yield measurably less base than a richer soil.

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BACTERIA DECOMPOSING ALGINIC ACID[1]

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Alginic acid is a complex carbohydrate belonging to the group of polyuronides; these yield, on hydrolysis with mineral acids, sugar acids, known as uronic acids, or mixtures of these acids with hexose or pentose sugars; purified alginic acid yields on hydrolysis only mannuronic acid. Alginic acid occurs abundantly in various marine algae, to the extent of 20 to 30 per cent of the total algal material. Although algal residues serve as food for a great variety of animals living both in water and on land, a large part of the residues are decomposed in nature by the action of microörganisms. The processes of their decomposition, especially the fate of the alginic acid complex, still remain to be determined.

In the course of a study of the decomposition of marine algal material, a number of bacteria capable of decomposing alginic acid were isolated from sea water, from phytoplankton and from the sea bottom. The decomposition of this compound was also studied in the case of land soils and several bacteria isolated; these differed, however, in certain of their physiological characteristics from the marine bacteria. It is important to add here that bacteria are the all-important agents concerned in the decomposition of alginic acid in nature. Even in soil, which is such a favorable medium for the growth of numerous fungi and actinomyces, the decomposition was carried out almost entirely by bacteria. The most active cellulose- and hemicellulose-decomposing fungi

[1] Contribution No. 43 of the Woods Hole Oceanographic Institution and Journal Series Paper of the New Jersey Agricultural Experiment Station, Department of Soil Microbiology.

present in the soil were found to attack the alginic acid only to a limited extent. This was further substantiated by the fact that while active specific enzyme preparations were obtained from the bacteria, fungus enzymes, such as taka-diastase and pectolytic enzymes, had no effect upon the alginic acid.

The following methods were used in the isolation of the bacteria from their respective habitats. One per cent solution of purified alginic acid (Schmidt and Vocke, 1926) prepared from different species of Fucus, largely *F. vesiculosus*, was dissolved in NaOH or KOH solution and the reaction adjusted to pH 7.0 to 7.2. Sodium nitrate and salts, in concentrations used in Czapek's solution were added. For the isolation of the marine bacteria, sea water or 3.5 per cent NaCl solution was used; for soil bacteria, distilled water.

The liquid media were inoculated with sea water, a piece of algal growth, phytoplankton, marine mud or sand, or with land soil. After bacterial development took place, as shown by the formation of turbidity in the medium, by pellicle or gas formation, the crude cultures were plated out on the corresponding agar media, and the bacteria isolated. A number of cultures were obtained; these were tested for their ability to decompose alginic acid, and only the most active forms selected for further study.

The determination of the decomposition of alginic acid by bacteria presents no difficulty in the case of distilled water or salt water media; the residual alginic acid is precipitated by means of a mineral acid or by addition of a solution of a calcium salt. In the case of the sea water medium, a large part of the alginic acid is precipitated in the medium by the calcium and magnesium of the water; as a result of the development of specific bacteria, the precipitate gradually disappears, due both to the action of the organisms and to that of the enzyme, alginase, which they produce abundantly.

The various alginic-acid-decomposing bacteria so far isolated both from the sea and from soil were found to be Gram-negative, non-spore-forming rods; they vary, however, considerably in their physiological properties. Some are more specific than others: some are able to attack, in addition to alginic acid, starch

and other carbohydrates and even agar-agar, while others are more strictly limited to alginic acid as the chief source of energy. The soil bacterium described here produces gas actively from alginic acid, while the marine bacteria do not. Some of the bacteria produce pigments, while others do not. They also vary in the rapidity of alginic acid decomposition. All the bacteria stain better with aqueous fuchsin or gentian violet than with carbol erythrosin; with the latter stain, the cells appeared much smaller and more coccoid.

A description of four types of bacteria selected as representing the alginic-acid-decomposing organisms in nature is reported here. The forms most closely related to this group are the agar-liquefying bacteria. The *B. gelaticus* of Gran and especially the form later described by Lundestad (1928), under the name of *B. granii*, seem to be related to the first organism, namely *Bact. alginovorum*. However, the growth characteristics of the two organisms are sufficiently different and the activity of the latter in decomposing alginic acid is so marked that a detailed description of this form is justified. The *Bact. fucicola*, with its specific characteristics and pigment production, has evidently not been described before. *Bact. alginicum* may have been referred to in the various descriptions of marine, especially agar-liquefying, bacteria, such as those of Angst (1929), but because of the fact that these descriptions are so imperfect, it was felt best to describe this form as well.

BACTERIUM ALGINOVORUM N. SP.

Rods with rounded to almost elliptical ends, especially when single. Occurring frequently in twos and even in chains, 1.5 to 2.0 by 0.75 to 1.2 microns in size. Non-spore-forming. Actively motile. Gram-negative. Capsule-forming. Stains well with aqueous fuchsin. Appears as a short coccoid form in carbol erythrosin stain.

Alginic acid plate: colony large, white in appearance with coarse granular center, entire margin. Clears up turbidity caused by the alginic acid on plate. No odor.

Alginic acid liquid medium: heavy pellicle formation. Active

production of an enzyme, alginase, which brings about the disappearance of the alginic acid precipitate in sea water medium. On salt water medium, a slimy pellicle of a highly tenacious nature is produced, the whole medium later turning to a soft jelly.

Sea water gelatin: active and rapid liquefaction in two to six days, at 18°C.; highly turbid throughout the liquefied zone.

Sea water glucose broth: abundant uniform turbidity, with surface pellicle; some strains give heavier turbidity and others heavier pellicle.

Litmus milk containing 3.5 per cent salt: no apparent growth.

Potato moistened with sea water: moist, spreading growth, ivory-colored; heavy sediment in free liquid at bottom.

Starch plate: abundant, cream-colored slimy growth; extensive diastase production.

Agar liquefaction: extensive softening of agar, no free liquid.

Aerobic, although able to grow under reduced oxygen tension.

Optimum temperature: 20°C.

Habitat: isolated from sea water, sea bottom sediments and from surface of algal growth in the sea. Very common in the sea.

BACTERIUM ALGINICUM N. SP.

Rods short to almost spherical, 0.6 to 1.0 micron in diameter. Non-spore-forming. Sluggishly motile. Gram-negative. Capsule-forming. When stained with aqueous fuchsin, the organism is found to be definitely a rod with rounded ends, occurring in twos. In carbol erythrosin stain, it is distinctly coccoid.

Alginic acid plate: white, finely granulated colonies, with entire margin. Does not clear up the turbidity in plate, due to limited production of the enzyme alginase. Odor formed, resembling that of old potatoes.

Alginic acid liquid medium: thin pellicle, weak alginase formation. Grows less rapidly and decomposes alginic acid less actively than previous organism.

Sea water gelatin: thin growth throughout gelatin stab, no liquefaction in seven days at 18°C.

Sea water glucose broth: uniform but very limited turbidity; no pellicle; no sediment.

Litmus milk containing salt: no apparent growth.

Potato moistened with sea water: moist, spreading growth, cream-colored; heavy sediment in free liquid at bottom.

Starch plate: limited, pale blue growth; no diastase.

Agar liquefaction: none.

Aerobic.

Optimum temperature: 20°C.

Habitat: isolated from sea water, and from surface of algal growth. A common form.

BACTERIUM FUCICOLA N. SP.

Rods, short, with ends rounded to almost coccoid; slightly curved; occurs singly and in twos. Does not stain evenly, 1.0 to 1.5 by 0.6 to 1 micron in size. Gram-negative. Actively motile, with twirling motion.

Alginic acid plate: colonies finely granular, entire; at first whitish, turning in three to five days brown, and later almost black, producing a deep brown soluble pigment.

Alginic acid liquid medium: limited growth on surface in the form of a pellicle. Frequently produces no growth at all.

Sea water gelatin: active liquefaction; no growth in stab; thin, fluorescent growth throughout liquefied zone.

Sea water glucose broth: faint turbidity; no pellicle, no sediment.

Litmus milk containing salt: no apparent growth.

Potato moistened with sea water: no growth.

Starch plate: no growth.

Agar liquefaction: positive, although limited; only softening of agar.

Aerobic.

Optimum temperature: 20°C.

Habitat: isolated from sea water taken from George's Bank, near the surface of the sand bottom. Rare occurrence.

BACTERIUM TERRESTRALGINICUM N. SP.

Long rods, with somewhat rounded ends, occurring usually single, but also in twos, and occasionally in chains of shorter rods,

1.5 to 2.5 by 1.0 to 1.5 microns. Gram-negative. Motile. Granular.

Alginic acid plate: colonies small, whitish in appearance with a slight metallic sheen.

Alginic acid liquid: medium at first clouded. Later, a pellicle is formed on surface of medium, which is soon broken up due to active gas formation. Reaction of medium becomes slightly alkaline.

Gelatin medium: slow growth throughout stab; slow liquefaction at surface of medium, at 18°C.

Glucose broth: abundant turbidity; some sediment; no pellicle; slightly fluorescent.

Litmus milk: acid; milk coagulated; only limited digestion of coagulum.

Potato: abundant, pinkish-colored, compact, dry growth on surface of plug, the rest of plug becoming grey, with tendency to darkening.

Starch plate: limited growth along streak; no diastase.

Agar liquefaction: none.

Aerobic to facultative anaerobic.

Optimum temperature: 30°C.

Habitat: soil.

In order to illustrate the action of the different bacteria upon alginic acid and upon crude algin, certain data are reported in tables 1 to 3. The term algin is applied to the washed acid precipitate of the dilute ammonium hydroxide extract of the algal material. The crude algin preparation contained 51.8 per cent uronic acid, 2.1 per cent ash and 1.66 per cent nitrogen.

In the first experiment, 100-cc. portions of sea water medium containing 1 gram of the air-dry alginic acid preparation, in the form of a potassium salt, were placed in flasks. A stream of CO_2-free air was passed above the cultures. The CO_2 produced by the organisms was absorbed in standard $Ba(OH)_2$ solution and the excess of the latter titrated with standard oxalic acid. Decomposition of the alginic acid was allowed to proceed, at room temperature, for twenty-one days. The results (table 1) show that the purified alginic acid is decomposed by the various bac-

teria. However, the extent of decomposition of the alginic acid in the crude algin preparations was much less, with greater varia-

TABLE 1

Decomposition of purified alginic acid by marine bacteria

Incubation 21 days

BACTERIAL CULTURE	CO_2 GIVEN OFF	RESIDUAL ASH-FREE ALGINIC ACID	ALGINIC ACID DECOMPOSED
	mgm. of C	*mgm.*	*mgm.*
Control	0.15	935	
Bact. alginicum	113.93	308	627
Bact. alginovorum, No. 4	98.63	160	775
Bact. alginovorum, No. 10	101.56	150	785
Bact. fucicola	122.13	175	760

TABLE 2

Decomposition of crude algin by marine bacteria

Incubation 21 days

BACTERIAL CULTURE	CO_2 GIVEN OFF	RESIDUAL ASH-FREE ALGIN	ALGIN DECOMPOSED
	mgm. of C	*mgm.*	*mgm.*
Control	0.40	266	
Bact. alginicum	43.21	181	85
Bact. alginovorum, No. 4	56.86	118	148
Bact. alginovorum, No. 10	44.92	146	120
Bact. fucicola	46.26	156	110

TABLE 3

Decomposition of alginic acid by Bact. terrestralginicum

	URONIC ACID ANHYDRIDE		REACTION OF CULTURE
	Left	Decomposed	
	mgm.	*mgm.*	*pH*
Control	443.5		6.5
Bacterium	343.3	100.2	7.2

tion among the different bacteria (table 2). In the last experiment, 300-mgm. portions of the algin preparation in the form of the potassium salt were used in 100-cc. portions of medium.

The rapidity of decomposition of the alginic acid by the soil organism was then tested. Fifty-cubic centimeters of distilled water medium, each containing 0.5 gram of the air-dry alginic acid preparation, in the form of a sodium salt, were inoculated with the bacterium and incubated at 28°C. for twenty days. No attempt was made to measure the CO_2 given off in the growth of this organism. The amount of uronic acid anhydride decomposed by the culture was determined. However, the decrease in total uronic acid content can not be taken as a true index of decomposition of the original alginic acid, since considerable hydrolysis of complex to smaller mannuronic acid units took place, without complete decomposition. The growth of the soil organism was accompanied by active gas evolution.

The various bacteria, both of marine and soil origin, were also found capable of decomposing alginic acid in the original Fucus material.

SUMMARY

The decomposition of alginic acid, a polyuronide occurring abundantly in marine algae, was found to be brought about largely by the action of certain specific bacteria, and only to a very limited extent by other microörganisms, such as fungi. Specific bacteria capable of decomposing alginic acid occur abundantly in sea water, in marine plankton, in the sea bottom and in land soils.

Four species of bacteria are described in this paper, three of which were isolated from the sea and one from the soil.

These bacteria decompose alginic acid actively, not only in purified preparations, but also in the original algal material.

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THE PRESENCE OF NITRIFYING BACTERIA IN DEEP SEAS[1]

PROBABLY no other phase of marine bacteriology has attracted as much attention as the process of oxidation of ammonium salts, which results in the formation of nitrite, followed by the oxidation of the nitrite to nitrate. Some investigators believed that the cycle of nitrogen in the sea is practically similar to that in land soils, while others were convinced that nitrates are not formed in the sea itself but are brought into the sea from the atmosphere or by land drainage; still others denied altogether the bacterial nature of the process and suggested its origin by electric discharges or by photochemical processes.

The work of Brandt, Thomsen, Issatchenko and Lipman led, however, to a definite conclusion that, while nitrifying bacteria may be absent in sea water, they are present abundantly in the sea bottom. Issatchenko[2] demonstrated the presence of these organisms in the North Arctic Ocean and in the Sea of Azov; he found them also in the Black Sea, but only in the bottom material close to shore. Similar results were obtained at the Woods Hole Oceanographic Institution,[3] for samples taken from the Gulf of Maine and from George's Bank; the method used for demonstrating the presence of these organisms was found to be of primary importance.

[1] Contribution No. 44 of the Woods Hole Oceanographic Institution and Journal Series Paper, New Jersey Agricultural Experiment Station, New Brunswick, N. J.

[2] B. Issatchenko, "Sur la nitrification dans les mers." *Compt. Rend. Acad. Sci.*, 182: 185–186, 1926.

[3] S. A. Waksman, M. Hotchkiss and C. L. Carey. "Marine Bacteria and Their Rôle in the Cycle of Life in the Sea. II. Bacteria Concerned in the Cycle of Nitrogen in the Sea." *Biol. Bull.*, 65: 137–167, 1933.

One of the major criticisms[4] directed against the findings of the nitrifying bacteria in the sea only along the littoral zone was that they are not normal sea inhabitants, but that they are rather a result of introduction from land soils by means of drainage waters. In order to meet this criticism, samples of sea-bottom material were obtained on the oceanographic vessel, *Atlantis,* from deep seas, north of Bermuda. The greatest depth of the water was 4,742 meters. The methods used for obtaining the samples of sea bottom under sterile conditions as well as for determining the presence of the specific organisms are described in detail elsewhere.[5] Active formation of nitrite, in an ammonium sulfate medium, was obtained at room temperature in 7 days, in the case of some samples, and in 27 days in all samples, as shown in Table I. It may be of interest to add that when cul-

TABLE I

Station No.	Depth of sea bottom, meters	Location		Nitrite-formation in days*				
		Lat.	Long.	7	18	27	36	90
1725	165	40°01′N	70°04′	0	0	tr	0	++
1726	643	39°51′	69°59′	tr	tr	+	+	++
1730	3,220	38°47′	69°11′	tr	+	+	+	+
1736	4,742	36°50′	68°52′	tr	+	+	++	++

* 0 = negative; tr = trace of nitrite; + = positive nitrite test; ++ = abundant nitrite formation.

tures from stations 1730 and 1736 were incubated at 8° C., active nitrite formation from ammonium salt was obtained in 46 days.

Although the presence of nitrite-forming bacteria was demonstrated in practically all the samples of marine-bottom material, no matter at what depth and

[4] H. H. Gran, "Bacteria of the Ocean and Their Nutrition." *Naturen* (Bergen), 27: 33, 1903.

[5] Waksman, Hotchkiss and Carey, *loc. cit.*

at what distance from shore, the formation of nitrate could not be demonstrated in the cultures. This is due to the fact that the method of testing for nitrate is much less sensitive than that for nitrite, and also because the nitrate-forming organisms develop much later. In the case of the cultures of nitrifying bacteria obtained in 1932 from the bottom deposits taken off Gay Head, it took from 42 days to nearly three months' incubation of the cultures before the nitrate-forming bacteria could be demonstrated. However, once they began to develop in the medium, further transfers resulted in active production of nitrate within a week or two.

These results prove beyond any doubt that the process of nitrification in the sea is brought about by bacteria which are native inhabitants of the sea bottom and are not merely contaminations from land.

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THE PENETRATION OF THE BLUE AND RED COMPONENTS OF DAYLIGHT INTO ATLANTIC COASTAL WATERS AND ITS RELATION TO PHYTOPLANKTON METABOLISM

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The following observations were undertaken as a continuation of the investigation of the conditions of submarine irradiation in relation to the biology of marine organisms. The measurements reported here and previously (Clarke, 1933) on the penetration of daylight into Atlantic waters serve as a basis for a comparison of this region with others which are being investigated intensively (Atkins and Poole, 1933; Utterback, 1933; Utterback and Boyle, 1933; Richardson, 1933; Erikson, 1933; Pettersson, 1934; and Pettersson and Landberg, 1934). The present measurements were made at two stations in the Gulf of Maine, one in the deep central part and one on Georges Bank, and at four localities in the region of Woods Hole. At the stations in the Gulf of Maine the vertical distribution of the phytoplankton was investigated (Braarud, 1934), complete hydrographic observations were made, and the diurnal migrations of copepods during the entire periods were studied (Clarke, 1934). Detailed information on the irradiation present at various depths is valuable not only in its immediate application to these studies of the plankton but also as an essential part of an intensive study of the oceanography of the Gulf of Maine (*cf.* Rakestraw, Gran, and Waksman, 1933). The observations in the neighborhood of Woods Hole were made in Woods Hole Harbor, Buzzards Bay, Vineyard Sound, and off Gay Head—localities chosen as representing four typical environments in this region. It is hoped that these measurements of light penetration will be useful in relation to certain of the biological problems being studied at Woods Hole, particularly those dealing with ecological relationships (*cf.* Allee, 1934). Some measurements of the photosynthesis of diatoms in this region are reported at the end of this paper.

[1] Contribution No. 45.

Apparatus and Methods

The photoelectric method previously described (Gall and Atkins, 1931; Clarke, 1933) was used. For the measurement of the blue component of daylight (3460Å–5260Å) the same deck photometer was employed. For submarine measurements the gas-filled potassium (sensitized) photoelectric cell was used in the water-tight case equipped with the four-conductor cable and the internal shutter. This combination is designated in the tables as Photometer 1*a*. The high sensitivity of the gas-filled cell made it useful for measurements at the deeper levels, but more trouble with changes of emission—especially near the surface—was experienced than with the vacuum cell. The cell was always glowed before each reading and all the other precautions previously described were taken in these observations.

For measurements in the red region of the spectrum two Type PJ–14 vacuum cæsium on cæsium oxide cathode photocells were used (kindly furnished by the General Electric Co.) which have peaks of maximum sensitivity in the near ultra-violet and red regions of the spectrum (Fig. 1). One of these cells was mounted in a newly constructed deck photometer and the other in sea photometer case No. 2—this latter combination being designated in the tables as Photometer No. 2*b*. To limit the radiation reaching the cells, each was mounted under a Schott-Jena Type RG–1 glass filter, 5 cm. square and 2 mm. thick, which cut out all radiations shorter than 6000Å, and a water-cell 2 cm. in depth and containing a 1.5 per cent solution of $CuCl_2$ which absorbed radiations of wavelength longer than 7000Å (*cf.* Coblentz, 1911). The filters were placed against the inside surface of the photometer window, the spaces between the filters and between the upper filter and the window being filled with Canada balsam to minimize reflection loss at the glass faces. When examined spectroscopically the duplex filters were found to have a sharp cut-off at 6000Å and a graded cut-off at 7000 to 7100Å with approximately 3 per cent transmission at 7000Å.

The spectral sensitivities of the cæsium photocells without the diffusing disc and filters were determined by the General Electric Research Laboratory under the direction of Dr. L. R. Koller (Fig. 1). The photocells mounted in the photometer cases under the filters were standardized for the spectral range 6000Å–7000Å by the same method as that employed for the blue-sensitive photometers (Clarke, 1933).

No changes in emission due to "photoelectric fatigue" were observed in moderate steady light in using the cæsium cells, but after continued exposure to bright sunlight on the deck of the ship decreases in the sensitivity of these cells were found amounting to 14 per cent. The

presence of " dark currents " in the deck photometer required frequent readings of the response with the photometer shutter closed during a series of observations. All measurements below the surface have been corrected for " external reflection " and " internal reflection " by multiplying by the factor 1.09 as before.

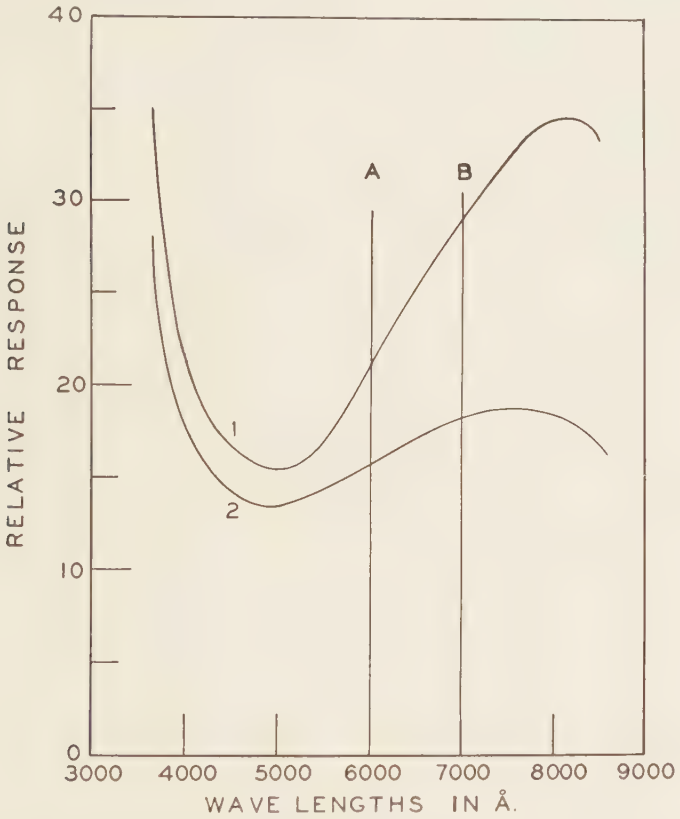

Fig. 1. Comparison of spectral sensitivities of red-sensitive photocells. (General Electric Research Laboratory.) Curve 1. No. 31,613. Cæsium on cæsium oxide—sea cell. Curve 2. No. 31,797. Cæsium on cæsium oxide—deck cell.

Each curve represents the relative response to an equal energy spectrum of the photocell alone.

In practice the cells are considered to be sensitive to the range between the vertical lines *A* and *B* as limited by the red RG–1 filter and the $CuCl_2$ filter cell.

Observations

The observations made on the penetration of blue and red light are summarized in Tables I and II. Curves showing the percentage of the

TABLE I

Summary of Observations in the Gulf of Maine

	Blue (3460Å–5260Å)			Red (6000Å–7000Å)			
Series No.	201	202	203	204	205	206	207
Station No.	1722	1722	1723	1722	1722	1723	1723
Lat. N.	42°50′	42°50′	41°19′	42°50′	42°50′	41°19′	41°24′30″
Long. W.	67°05′	67°05′	66°40′	67°05′	67°05′	66°40′	66°42′30″
Date, 1933	7/15	7/16	7/17	7/15	7/16	7/17	7/18
Local sun time (24-hr. scale)	1425–1515	1230–1440	1200–1410	1230–1340	1030–1200	1430–1535	1210–1325
Av. altitude of sun	48°	59°	63°	65°	64°	47°	56°
Photometer No.	1a	1a	1a	2b	2b	2b	2b
Av. transmissive exponent, *k*, over depth studied	0.11	0.11	0.14	0.31	0.32	0.35	0.34
Irrad. at depth of 1 meter (% surface light)	77	89	64	43	53	52	46
Depth at which irrad. is reduced to 1%, *meters*	40	34	26	12	12	11	12
Range of irrad. on deck during series *microwatts/cm.*^{2}	4,500–8,200	5,600–8,600	11,900–13,400	13,900–12,200	10,700–8,700	11,000–5,600	10,000–11,400
Sea (Douglas sea scale)	Slight	Smooth	Smooth heavy swell	Slight	Smooth	Smooth heavy swell	Slight heavy swell
Sky	Many clouds	Over-cast, fog	Clear, fog	Few clouds	Over-cast, fog	Clear, fog	Clear, fog

Table II

Summary of observations in the Woods Hole region. Woods Hole harbor, Series 122 and 211: bottom at 23 meters, tide flooding, *i.e.* water flowing in from Buzzards Bay. Off Gay Head, Series 208 and 212: bottom at 30 meters, current running west until 1537. Buzzards Bay, Series 209 and 213: bottom at 15 meters, high tide at New Bedford at 0920. Vineyard Sound (halfway between Nobska Point and West Chop), Series 210 and 214: bottom at 22 meters, current running east until 1500.

	Blue (3460Å 5260Å)				(Red (6000Å–7000Å)			
Series No.	122	208	209	210	211	212	213	214
Location	W. H. Harbor	Off Gay Head	Buzzards Bay	Vineyard Sound	W. H. Harbor	Off Gay Head	Buzzards Bay	Vineyard Sound
Lat. N.	41°31′	41°20′	41°33′	41°30′	41°31′	41°20′	41°33′	41°30′
Long. W.	70°40′	70°57′	70°42′25″	70°36′	70°40′	70°57′	70°42′25″	70°36′
Date	Aug. 22 1932	Sept. 2 1933	Sept. 6 1933	Sept. 8 1933	Aug. 30 1933	Sept. 2 1933	Sept. 6 1933	Sept. 8 1933
Local sun time (24-hr. scale)	1339–1429	1320–1445	1011–1210	1040–1145	1410–1540	1150–1305	1215–1350	1310–1450
Av. altitude of sun	50°	47°	54°	54°	40°	56°	53°	46°
Photometer No.	1	1a	1a	1a	2b	2b	2b	2b
Av. transmissive exponent, k, over depth studied	0.49	0.27	0.51	0.36	0.62	0.37	0.61	0.59
Irrad. at depth of 1 meter (% surface light)	48	79	50	47	29	39	43	42
Depth at which irrad. is reduced to 1%, *meters*	8	16	10	12	6	10	7	8
Range of irrad. on deck during series, *microwatts/cm.*^{2}	11,800–12,400	14,000–11,000	12,000–9,000	14,000–13,000	7,950–5,800	14,250–13,400	11,050–9,400	11,500–10,200
Sea (Douglas sea scale)	Calm	Smooth to slight	Smooth to slight	Smooth	Calm	Smooth to slight	Smooth	Slight
Sky	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear

surface light reaching the various depths for all the series appear in Figs. 2 to 5. Considering first the penetration of blue light, we find that the average transparency at Station 1722 in the Gulf of Maine is slightly greater than that at Station 1053 (Clarke, 1933[2]). This is to be expected since Station 1722 is nearer the open ocean. The average transparency on Georges Bank (Station 1723), where the shallower water may be stirred down to the bottom, is about the same as at Stations 1285,

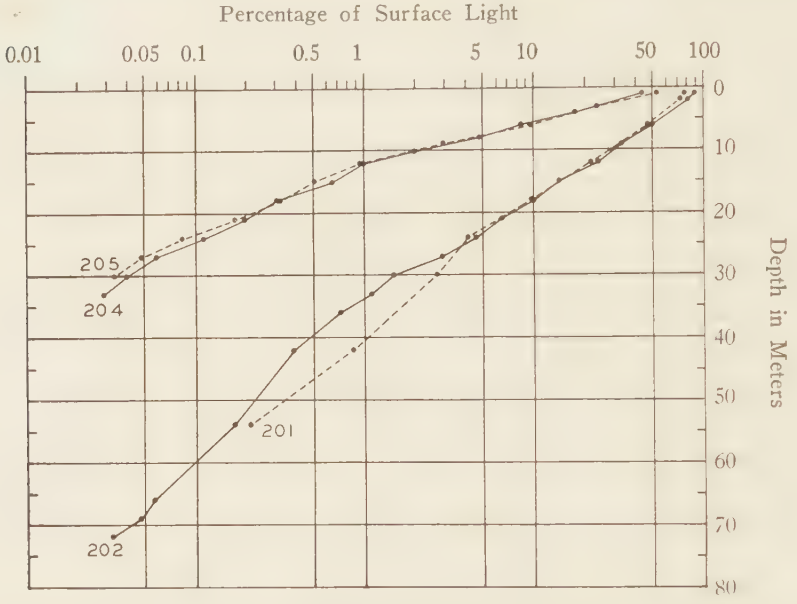

Fig. 2. Relation between depth and irradiation expressed as a percentage of the light just over the surface (logarithmic scale). Series number indicated at the end of each curve.

Station 1722: Gulf of Maine, deep basin.
Series 201 and 202—blue light.
Series 204 and 205—red light.

1286 and 1287 (previously reported) in deeper water but much nearer shore. The transparency of the water for blue light is from 2 to 4½

[2] The following corrections should be made in "Average altitude of sun" in Tables II and III, Clarke, 1933 Biol. Bull., vol. 65, p. 317.

Table II

Series No.	1	2	3	4	5	6	7	8	9	10	11	12	13
Av. altitude of sun	58°	54°	58°	61°	62°	64°	65°	65°	62°	61°	24°	67°	54°

Table III

Series No.	13	112	113	114	115	116	117	118	119	120	121	122
Av. altitude of sun	54°	62°	1°	28°	37°	41°	68°	6°	22°	58°	57°	50°

times lower in the Woods Hole region than in the Gulf of Maine. The water of Buzzards Bay (Series 209) was found to have a slightly greater average transmissive exponent than the water of Woods Hole Harbor (Series 122). However, it is the turbidity of the water layer just over the bottom in Buzzards Bay which reduces the average transparency for the whole series, the upper strata being somewhat more transparent. In Woods Hole Harbor this situation is just reversed, the surface water being slightly less transparent than the deeper strata. The result is

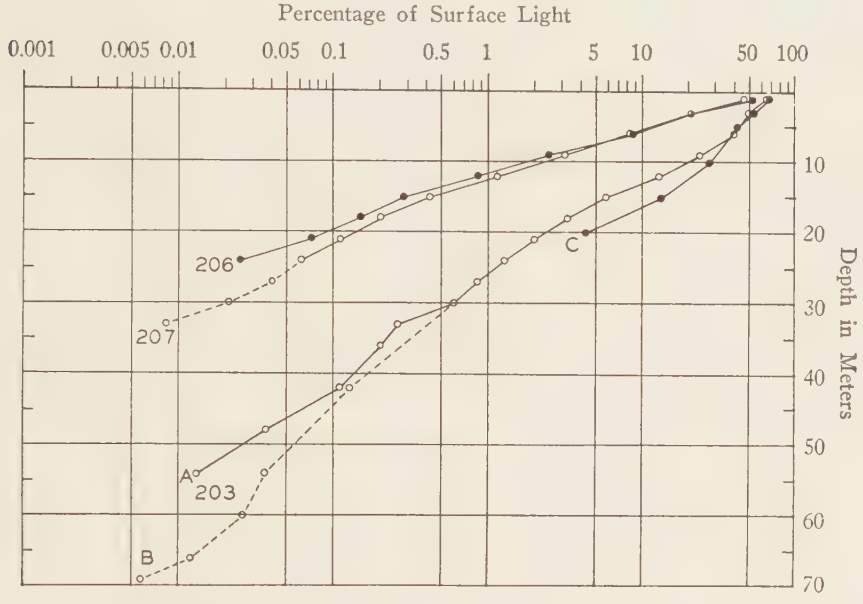

Fig. 3. Station 1723. Georges Bank.
Series 203—blue light.
a. Regular measurement.
b. Measurement of deeper strata with opal glass removed from photometer.
c. Measurement of upper strata repeated following day.
Series 206 and 207—red light. Broken portion of curve 207 represents measurement with opal glass removed.

that daylight penetrates to a depth of 10 meters in Buzzards Bay before it is reduced to 1 per cent of its surface value whereas a corresponding reduction has taken place in the Harbor at 8 meters. The water off Gay Head (Series 208) is about half as clear as that characterizing the Gulf of Maine, and the transparency in Vineyard Sound (Series 210) is intermediate between that found off Gay Head and in Buzzards Bay. The Gay Head and Vineyard Sound regions correspond rather closely to the San Juan Archipelago in transparency to blue light (Utterback and Boyle, 1933).

The average transparency for red light in the deeper part of the Gulf of Maine (Station 1722) is slightly higher than that found by Atkins and Poole (1933) off Rame Head, England, although there is a closer agreement when only the range from 1 to 10 meters is used for comparison. On Georges Bank (Station 1723) the transparency for red light falls between that found off Rame Head and in Whitsand Bay, for the range 1 to 5 meters, and is lower than at Station 1722. The penetration of red light into the water off Gay Head (Series 212) is only slightly lower than on Georges Bank and is higher than would be ex-

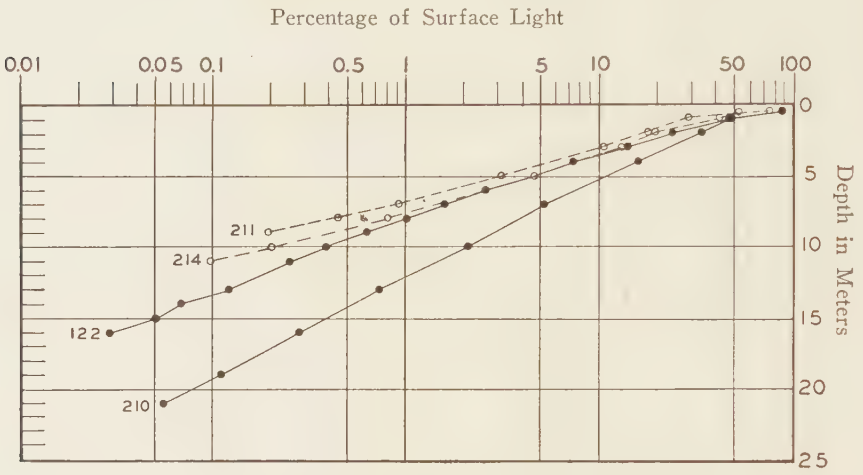

Fig. 4. Series 122. Blue light. Woods Hole Harbor.
Series 210. Blue light. Vineyard Sound.
Series 211. Red light. Woods Hole Harbor.
Series 214. Red light. Vineyard Sound.

pected for the locality. The waters of Vineyard Sound (Series 214), Buzzards Bay (Series 213), and Woods Hole Harbor (Series 211) are all very similar in transparency, but there is a slight increase in turbidity in the order mentioned. The transparency is approximately one-half that found at Station 1722.

In some of the series the transmissive exponent in the red decreases with depth. This is especially evident at Stations 1722 and 1723 and to a lesser extent in Series 212 where the depth of the water allows for some "layering." Atkins and Poole (1933) found similar results for certain observations in British waters, and Utterback and Boyle (1933) for measurements made in the waters of the San Juan Archipelago. Irradiation in the deeper layers is probably at the shorter wave-

lengths so that the energy reaching the photocell through the Schott RG–1 filter would be at or near to 6000Å, *i.e.* in the orange.

A comparison of the energy in the blue and red regions of the sun's spectrum is given in Fig. 6 as measured on the deck of the ship over the period of daylight on July 16, 1933 at Station 1722. For the spectral limits measured the agreement in the amount of energy is close. Since the sky during the period covered was overcast with some fog, the red radiation was probably transmitted more readily than the blue due to more scattering at the shorter wave-lengths. Scattering of blue light may also help to explain the close agreement at sunset when the red component would normally have the higher value.

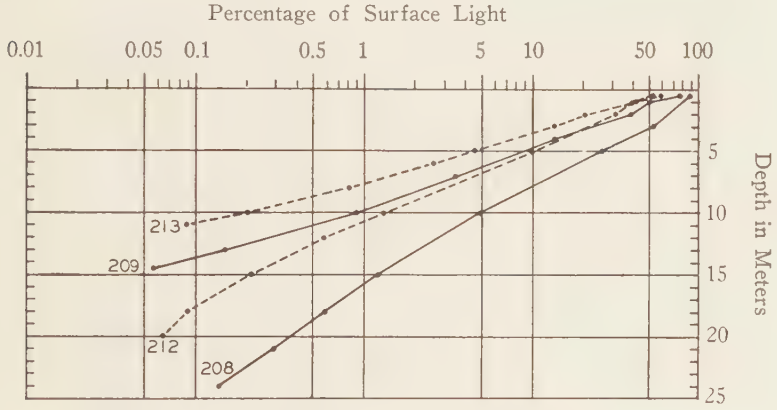

Fig. 5. Series 208. Blue light. Off Gay Head.
Series 209. Blue light. Buzzards Bay.
Series 212. Red light. Off Gay Head.
Series 213. Red light. Buzzards Bay.

DISCUSSION

The curves for the penetration of both blue and red light show marked differences in their degree of irregularity. Thus the curves for the Gulf of Maine are quite jagged in comparison with those for the Woods Hole region. This may be due in part to the much rougher condition of the sea experienced in the Gulf of Maine. It seems probable, however, that a more important factor is the thorough stirring caused by the very strong tidal currents of the Woods Hole region. Temperature measurements (such as those in Table III) show that the water is very nearly homogeneous from top to bottom. The irradiation measurements obtained in Vineyard Sound (*e.g.* Series 210), famous for its strong tide, fall on a remarkably straight line. Moreover,

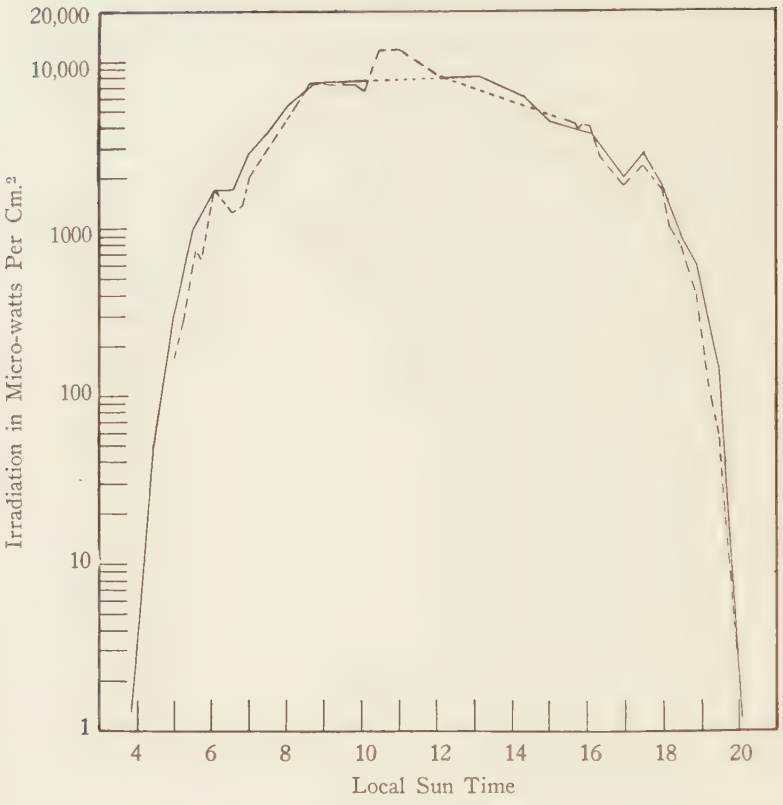

FIG. 6. Comparison of the blue and red components of daylight measured on deck during July 16, 1933 at Station 1722. Solid line indicates blue light, broken line indicates red light.

this regularity indicates that the method of measurement is highly reliable and that the irregularities in the values obtained at other localities are not to be assigned to fluctuation of the photometers.

If changes in the slopes of the curves really are caused by water layers of different transparency, it is difficult to understand why changes in the blue curve are not represented by corresponding changes in the red curve for the same locality, and vice versa, since the two sets of measurements were ordinarily taken within two hours of each other. Examples of such lack of conformity are seen, particularly, in the Gulf of Maine Series. The presence in different water layers of material having a highly selective effect on light would account for these irregularities, but the actual existence of such material has yet to be demonstrated. The differences between the three segments (*a*, *b*, and *c*)

of Series 203 certainly indicate a great lack of homogeneity in this case.

The relative clearness of the bodies of water considered is the same whether arranged in order of average transparency to blue light or to red light except for the Woods Hole Harbor and Buzzards Bay series. The transmissive exponent for blue light is slightly higher in Buzzards Bay than in Woods Hole Harbor, whereas the reverse is true for red light. However, the transparency of the harbor water is very close to that of the Bay water in both cases, and for blue light at least it is distinctly different from that of Vineyard Sound. This is in agreement with the fact that when the tide is flowing into the harbor, the water is derived chiefly from Buzzards Bay (*cf.* Fish, 1925).

Red light is absorbed faster than blue light in all of these bodies of water, but it is significant to note that the ratio of the transmissive exponents is not constant. The ratios of the exponents for red light to the exponents for blue light follow:

Station 1722	2.9
Station 1723	2.5
Off Gay Head	1.4
Vineyard Sound	1.6
Woods Hole Harbor	1.3
Buzzards Bay	1.2

It is seen that, in general, as the water becomes more turbid the difference between the penetration of blue and red light becomes less. This would seem explicable on the basis of the greater scattering effect of increasing amounts of suspended particles on radiation of short wave-length than on radiation of long wave-length. The ratio of the exponents for red and blue light in the Gulf of Maine is about the same as in Whitsand Bay (Atkins and Poole, 1933). The ratio in the Woods Hole region is similar to that in the San Juan Archipelago (Utterback and Boyle, 1933).

Note on the Metabolism of the Phytoplankton

Of previous investigations on the photosynthesis of aquatic plants in relation to the spectral distribution of the irradiation (*cf.* Klugh, 1930), few deal specifically with the marine phytoplankton. It is therefore difficult to correlate the present measurements of the penetration of light with the vertical distribution and metabolism of diatoms, etc. However, since photosynthesis is of primary significance in the economy of the sea, it seems important to report such observations as are available. Laboratory experiments indicate that intensity is more important than color for the photosynthesis of diatoms, but since chromatic adapta-

tion occurs, it is necessary to know the spectral composition as well as the total intensity of the irradiation present (Stanbury, 1931). Klugh (1930) concludes that certain pigments present in the cell may act as photo-sensitizers for the chlorophyll in plants growing at depths lower than the first few meters. Schreiber (1927) exposed various species of diatoms and dino-flagellates to general radiations and showed that the optimum radiant energy for growth differed widely in different forms.

On August 16 and August 22, 1932, Dr. H. H. Gran carried out measurements of the metabolism of diatoms in Woods Hole Harbor at the same time that the observations on the penetration of blue light were being made (Series 120, 121, and 122). Comparable values for red light may be obtained from Series 211. For the experiments sea water with the contained diatoms was taken from Vineyard Sound and placed in glass-stoppered bottles of 200 cc. capacity. The bottles were placed horizontally in shallow wire baskets and these were lashed at 3-meter intervals to a weighted rope hung from the south side of the anchored boat. Each basket contained two bottles fully exposed to light, and four of the baskets contained in addition one bottle which was completely covered with black cloth, thus providing an opportunity to measure respiration in the absence of photosynthesis. The oxygen present in two control bottles was measured, using the Winkler method at the beginning of the exposure period, and similar measurements were made on all the other bottles at the end. The bottles were exposed at their respective depths for about 4 hours during the middle of the day.

The experiments were not intended to yield more than approximations and the results are somewhat irregular. However, the location of the depth at which the oxygen consumed in respiration is just balanced by the oxygen evolved in photosynthesis (*i.e.* the "compensation point") seems quite definite. The results of the experiment on August 22 are presented in Table III, where it is seen that the bottles at the 3-meter and 6-meter levels showed an increase in oxygen whereas those at greater depths showed a decrease. Since on August 16 the shallowest bottle was placed at 8 meters and all the bottles showed a decrease of oxygen content at the end of the experiment, we may place the "compensation point" for Woods Hole Harbor at about 7 meters. At this depth the average irradiation present during the experiment was as follows:

Blue	192 microwatts/cm.^{2}
Red	108 microwatts/cm.^{2}

On June 1, 1934, Dr. A. C. Redfield carried out similar measurements, using the same methods, while the "Atlantis" was anchored at

Station 2212 in the Gulf of Maine 40 miles east of Cape Cod. A diatom haul made on Georges Bank was allowed to stand for 2 days and the suspended material decanted off and diluted. Bottles containing this diatom suspension were placed at 10-meter intervals down to 50 meters and left for 7 hours. When the tide was running strongly the bottles

TABLE III

Metabolism of diatoms in Woods Hole Harbor. Exposure period: 1002–1427, August 22, 1932. Range of irradiation on deck during exposure period: blue (3460Å–5260Å): 12,400–11,600 microwatts/cm.²; red (6000Å-7000Å): 12,000-11,200 microwatts/cm.² * Oxygen present before exposure: 6.74 cc./liter. Oxygen present in covered bottles at end of period: 6.64 cc./liter.

Depth	Temp.	Average irradiation blue	Average irradiation red	Oxygen after exposure	Change in oxygen
meters	° *C.*	*microwatts/cm.²*	*microwatts/cm.²*	*cc./l.*	*cc./l.*
3	21.40	1700	1240	7.06	+0.34
6	21.39	370	197	6.81	+0.07
9	21.33	98	23	6.72	−0.02
12	21.27	27	3	6.71	−0.03
15	21.26	7.2	—	6.73	−0.01
18	21.27	—	—	6.67	−0.07

*This value assumed from information obtained a year later on the ratio of the blue and red components of daylight.

were brought slightly nearer the surface, as is indicated in Table IV. The results are satisfactorily consistent and show that photosynthesis took place down to at least 40 meters. The progressive diminution of oxygen produced from the surface downwards is a result both of the decreased irradiation and of the lowered temperature. The amount of oxygen consumed in respiration likewise diminished with depth and is correlated with the temperature. The "compensation point" occurred at 30–24 meters.

Since no measurements of irradiation were made during these observations, it is impossible to make an exact comparison of the results with those obtained in Woods Hole Harbor. However, it is worth pointing out that in the Gulf of Maine the water is roughly three times as transparent as in Woods Hole Harbor and the "compensation point" lies about three times as deep. Marshall and Orr (1928) found the "compensation point" in Loch Striven to occur between 20 and 30 meters, but a satisfactory comparison with their observations is similarly difficult because of the different methods used. The calculation of light intensity for Loch Striven made by Atkins and Poole (1933) cannot readily be compared with ours since the values arrived at are for irradia-

tion over the whole spectrum throughout the day and ours are concerned with two limited regions of the spectrum for a few hours. It appears, however, that the water of Loch Striven, like the water in the Gulf of Maine, is somewhat more than three times as transparent as the water in Woods Hole Harbor and the "compensation point" for the whole day lies correspondingly deeper.

TABLE IV

Metabolism of diatoms in the Gulf of Maine. Exposure period: 0444–1354, June 1, 1934. Sky: cloudy, becoming clear and later overcast. Sea: smooth, moderate swell.
Oxygen present before exposure: 7.80
7.83
7.83

av. 7.82 cc. per liter.

Depth	Temp.	Condition of bottles	Oxygen after exposure	Change in oxygen	Oxygen produced
meters	° *C.*		*cc./l.*	*cc./l.*	*cc./l.*
0	10.75	covered	7.11	−0.71	
		exposed	9.42	+1.60	+2.31
		"	9.44	+1.62	+2.33
10–8	10.60	covered	7.32	−0.50	
		exposed	8.40	+0.58	+1.08
		"	8.44	+0.62	+1.12
20–16	10.0	covered	7.42	−0.40	
		exposed	8.07	+0.25	+0.65
		"	8.02	+0.20	+0.60
30–24	6–9	covered	7.61	−0.21	
		exposed	7.81	−0.01	+0.20
		"	7.72	−0.05	+0.16
40–32	5–6	covered	7.54	−0.28	
		exposed	7.66	−0.16	+0.12
		"	7.71	−0.11	+0.17
50–40	4–5	covered	7.61	−0.21	
		exposed	7.59	−0.22	+0.01
		"	7.64	−0.18	−0.03

From the samples of phytoplankton collected in July, 1933 at Stations 1722 and 1723 and subsequently subjected to a quantitative study by Mr. T. Braarud (1934) it was found that the dominant form as to numbers at both stations was the coccolithophoride *Pontosphæra Huxleyi,* which reached its maximum at approximately 18 meters at Station 1722

and at near 14 meters at Station 1723. Braarud (1934) states that the production of *Pontosphaera* seems to have been confined to the layer in which the temperature was higher than 10° C. At these depths where the maximum of *Pontosphæra* occurred the blue light was reduced to 9.9 per cent and 11.4 per cent respectively and the red light to 0.31 per cent and 0.50 per cent respectively. Taking 13,000 and 11,000 microwatts/cm.^{2} for approximate values of blue and red irradiation at the surface at noon on a clear day, we obtain light intensities of 1290 and 1480 μW/cm.^{2} for the blue and of 34 and 55 μW/cm.^{2} for the red for these depths. It will be noted that the blue irradiation is greater and the red irradiation less than that found at the "compensation point" in Woods Hole Harbor. However, no conclusions can be drawn from these facts as yet since the effect of the different parts of the spectrum on photosynthesis and the influence of other environmental factors are so poorly understood.

It is of interest to inquire whether the phytoplankton population has an appreciable effect on the reduction of light with depth (*cf.* Atkins and Poole, 1933). No sharp changes in the slopes of the curves occur at the levels of maximum abundance of *Pontosphæra.* The diatom, *Guinardia flaccida,* similarly has no noticeable influence upon the transparency. This species, being about fifty times larger than *Pontosphæra,* is the dominant form in respect to bulk at Station 1723 and occurs in maximum numbers at 30 meters.

It is evident that the whole question of the relationship between submarine irradiation and the metabolism of the phytoplankton must be much more thoroughly investigated both in the laboratory and at sea. In particular, for an understanding of the biology of Atlantic coastal waters we lack measurements of the penetration of the green and yellow parts of the spectrum. We now have a general idea of the vertical distribution of the plankton in these waters and of the conditions of submarine irradiation in respect to blue and red light.

Summary

1. Photoelectric measurements of blue and red submarine irradiation were made at two stations in the Gulf of Maine and at four localities in the Woods Hole region. The same apparatus was used as before (Clarke, 1933) with the addition of two cæsium on cæsium oxide photocells mounted under Schott-Jena RG–1 filters and $CuCl_2$ water-cells which limited the radiation received to 6000Å to 7000Å.

2. The bodies of water considered may be arranged in order of decreasing transparency to both blue and red light as follows: (*a*) the

deep basin of the Gulf of Maine, (*b*) Georges Bank, (*c*) Off Gay Head, (*d*) Vineyard Sound, (*e*) Buzzards Bay and Woods Hole Harbor. The ratios of the transmissive exponents for red light to those for blue light in each locality vary from 2.9 to 1.2, the higher values being characteristic of clearer water.

3. These measurements are discussed in relation to the local hydrographic conditions, and are compared with observations made in the Plymouth region and in the San Juan Archipelago.

4. Measurements made by Dr. H. H. Gran on the metabolism of diatoms in bottles suspended at different depths in Woods Hole Harbor indicated that the "compensation point" was located at a depth of about 7 meters. Similar measurements carried out by Dr. A. C. Redfield gave a depth of 24–30 meters for the "compensation point" in the Gulf of Maine.

5. Observations reported by Mr. T. Braarud (1934) on the vertical distribution of the phytoplankton at the same stations in the Gulf of Maine are discussed in relation to the penetration of light.

The authors desire to express their thanks to Mr. R. T. Montgomery for his kind assistance in the measurements made at sea.

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A NOTE ON THE PHYTOPLANKTON OF THE GULF OF MAINE IN THE SUMMER OF 1933

TRYGVE BRAARUD

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The rather scanty quantitative data on the phytoplankton distribution in the Gulf of Maine in summer are mostly from the western part, collected during 1931–32 for the International Passamaquoddy Fisheries Commission (Gran and Braarud, 1934). In addition, Gran (1933) studied the phytoplankton distribution in the summer of 1932, from material collected at several stations. The phytoplankton described in this paper was obtained from water bottle samples taken by Clarke at two stations in the Gulf of Maine during his work on the light conditions in the water and on the vertical migration of the zoöplankton. These stations represent much the same part of the Gulf as do two of the stations from which the 1932 material was collected. The 1932 observations were made on August 3–4, while those in 1933 occurred about two weeks earlier in the season, on July sixteenth and eighteenth. The present collections supplement our knowledge of the distribution of phytoplankton in this region and also provide data for the study of the relationships between the plant population and the penetration of daylight into the water. Since the latter aspect of the work is discussed by Clarke and Oster (1934) for these stations, I shall confine myself to a short account of the distribution of the phytoplankton. The question of the importance of diatoms in light absorption has been discussed by Poole and Atkins (1929) and by Marshall and Orr (1930).

The surface layers at Station 1722 (in the eastern part of the Gulf of Maine) consisted of presumably local water of low salinity (32.07 to 32.19‰), while at Station 1723 the uppermost layer was of Atlantic origin, having a salinity between 33.12 and 33.54‰. (See Table III.) In spite of this conspicuous difference in the characteristics of the water, the phytoplankton communities at the two stations had much the same character. The form numerically dominant at both localities was the coccolithophoride *Pontosphæra Huxleyi,* which was abundant at a depth of 1 to 25 or 30 meters. At both stations the maximum number was found at an intermediate depth (15, 18 meters), and the production of *Pontosphæra* seems to have been confined to the

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layer in which the temperature was more than 10° C. This is corroborated by the oxygen distribution, as the layer in which there was supersaturation of oxygen is the same as that in which the numbers of *Pontosphæra* were high. The only exception is Station 1723, where at 25 meters 30,000 cells of *Pontosphæra* per liter were found and yet there was no supersaturation in oxygen. Here the temperature was only 8.67° C. The general distribution of *Pontosphæra Huxleyi* seems clearly to indicate that it is a moderately thermophile form which reproduces very slowly at temperatures lower than 7° C. (Gran, 1930, p. 32). It seems probable, therefore, that this population at 25 meters was the result mainly of the sinking of the abundant population of *Pontosphæra* from the layers above (see Table III), and that the local production of *Pontosphæra* at this depth was small on account of the low temperature and the poor light conditions.

At Station 1723 (on Georges Bank) diatoms were fairly abundant, but only two species were recorded in any great numbers. In the upper warm layer *Detonula confervacea* occurred in small quantities in every sample, with a maximum number of 1,300 cells per liter at 15 meters—the same depth at which the maximum number of *Pontosphæra* occurred. *Guinardia flaccida* was distributed mainly below 20 meters, with a maximum of 5,300 cells per liter at 30 meters. This species has previously been recorded as a form which commonly, if not always, has occurred in the waters over the Bank (Bigelow, 1926; Gran, 1933). The *Ceratia* must have been very scarce at Station 1723, since in the centrifuged samples only a few specimens of *Ceratium longipes* and *C. tripos* were recorded. A colorless flagellate (*Bodo marina?*) was common at all depths.

At Station 1722 very few diatoms were observed, and no forms except *Pontosphæra Huxleyi* occurred in great numbers. The *Ceratia* were as scarce as at Station 1723. The *Guinardia* population was absent here, or at least present in numbers too small to be recorded in the volume of water which was centrifuged. Except for the *Guinardia*, the communities were very much the same in the two localities, but quantitatively poorer at Station 1722.

Although Gran's observations (1933) were not made in exactly the same localities as the present ones, an approximate comparison may be made between the summer vegetation at Georges Bank and in the eastern part of the Gulf of Maine in the years 1932 and 1933. The difference in time of season, viz., two weeks, is presumably of no great importance, since the conditions do not seem to change quickly at this time of the year. In the Gulf proper, a remarkable difference between the two years is found in the quality of the phytoplankton. In 1932 di-

Table I

Station 1722, July 16, 1933. Latitude, 42°50′ N, Longitude, 67°05′ W.

Depth in meters	1	6	12	18	30	42	54	66	78
Temperature, ° C	13.17	13.04	12.52	11.39	10.46	8.50	7.01	6.59	5.77
Salinity, ‰	32.07	32.07	32.09	32.11	32.19	32.42	32.63	32.75	32.80
Oxygen, cc. per liter	6.48	6.41	6.65	6.87	6.85	6.60	6.48	6.42	6.20
Oxygen, % saturation	107	105	108	109	107	99	94	92	88
PO_4', mg. per cu.m	29	29	29	29	30	55	60	66	78
NO_3', mg. per cu.m	11	13	9	8	11	55	80	85	104
Number cc. examined	10	10	10	10(50)	10(50)	10	10	50	50
Phytoplankton, cells per liter:									
Chætoceros sp	—	—	—	—	—	—	—	20	—
Corethron hystrix	—	—	—	—	—	—	—	20	—
Detonula confervacea	200	100	—	200	100	—	200	60	—
Coccolithus pelagicus	—	—	—	—	—	—	—	—	40
Pontosphæra Huxleyi	146,000	105,400	198,000	308,000	130,200	13,200	9500	6460	1880
Eutreptia viridus	—	—	—	200	—	—	—	—	—
Ceratium longipes	100	—	—	—	—	—	—	—	—
Exuviælla baltica	—	400	—	100	—	—	—	—	—
Gymnodinium Lohmanni	—	—	200	200	200	—	100	80	—
" sp	600	400	700	300	100	—	100	160	200
Minuscula bipes	—	—	—	100	—	—	—	20	—
Oxytoxum gracile	—	—	100	—	—	—	—	—	—
Peridinium sp	—	200	—	—	—	—	—	20	—
Acanthostomella norvegica	—	—	—	—	200	—	—	20	—
Laboea conica	—	—	—	100	—	—	—	20	—
" emergens	—	100	—	—	100	—	—	20	—
" reticulata	—	—	500	600	100	—	—	—	—
" strobila	—	—	—	100	—	—	—	—	—
Lohmanniella oviformis	200	300	—	800	2300	100	—	40	—
" spiralis	—	—	300	200	—	—	—	120	60
Mesodinium rubrum	—	—	100	—	—	—	—	—	—
Bodo marina?	6700	3800	9500	5300	5500	300	500	80	20
Forms not identified	1100	300	900	1900	3800	100	300	220	100

Table II

Station 1723, July 18, 1933. Latitude, 41°19′ N, Longitude, 66°40′ W.

Depth in meters	1	5	10	15	25	30	40	50	60
Temperature, ° C.	15.45	15.47	15.39	13.92	8.67	8.47	8.44	8.21	8.07
Salinity, ‰	33.12	33.15	33.54	33.27	32.51	32.53	32.56	32.56	32.60
Oxygen, cc. per liter	6.18	6.44	6.30	6.58	6.40	6.38	6.24	6.23	6.33
Oxygen, % saturation	107	112	110	111	97	96	94	93	94
PO_4, mg. per cu.m	28	31	28	49	65	68	76	72	75
NO_3, mg. per cu.m	14	11	8	9	53	54	67	62	62
Number cc. examined	25	10	10	10	10(25)	10	10	10	10
Phytoplankton, cells per liter:									
Chætoceros decipiens	—	—	—	—	—	900	500	—	—
Corethron hystrix	40	—	100	—	—	—	—	—	—
Coscinosira œstrupi	—	—	—	—	—	100	—	100	500
Detonula confervacea	400	600	600	1300	200	—	—	100	—
Guinardia flaccida	—	100	—	700	1800	5300	3700	3900	2200
Leptocylindrus danicus	—	—	—	200	800	—	—	—	—
Melosira sp.	—	—	—	—	—	100	—	—	—
" sulcata	—	—	—	—	—	—	400	—	—
Nitzschia closterium	40	—	—	—	—	—	100	100	100
" delicatissima	320	—	500	100	200	—	—	400	300
" seriata	—	—	—	—	—	200	—	—	—
Rhizosolenia alata	120	300	—		—	—	—	—	—
" fragilissima	40	—	—	—	—	100	—	—	—
" semispina	—	—	100	—	—	—	—	—	—
" styliformis	—	—	—	350	200	1500	—	800	50
Thalassiosira decipiens	—	—	—	—	—	200	—	—	—
" gravida				—	—	—	—	—	100
" sp.	—	—	200	—	—	—	—	—	—
Coccolithus pelagicus					200		—	—	—
Pontosphæra Huxleyi	67,490	50,500	64,200	120,000	30,000	9,100	1,800	2,300	1,400
Syracosphæra dentata	80	100	—	600		—		—	—
Eutreptia viridis	—	—	100	6500	600	—	—	—	—

TABLE II—*Continued*

Depth in meters	1	5	10	15	25	30	40	50	60
Temperature, ° C	15.45	15.47	15.39	13.92	8.67	8.47	8.44	8.21	8.07
Salinity, ‰	33.12	33.15	33.54	33.27	32.51	32.53	32.56	32.56	32.60
Oxygen, cc. per liter	6.18	6.44	6.30	6.58	6.40	6.38	6.24	6.23	6.33
Oxygen, % saturation	107	112	110	111	97	96	94	93	94
PO_4', mg. per cu. m	28	31	28	49	65	68	76	72	75
NO_3', mg. per cu.m	14	11	8	9	53	54	67	62	62
Number cc. examined	25	10	10	10	10(25)	10	10	10	10
Ceratium longipes	—	—	—	—	20	—	—	—	—
" tripos	80	—	—	—	—	—	—	—	—
Exuviælla baltica	120	300	200	—	—	—	—	—	—
Gonyaulax Tamarensis	—	100	—	—	—	—	—	—	—
Gymnodinium Lohmanni	480	300	400	200	100	—	—	—	—
" sp.	320	700	100	1500	300	—	—	—	100
Minuscula bipes	120	—	—	—	—	100	—	—	—
Oxytoxum gracile	440	200	200	800	—	—	—	—	—
Peridinium triquetrum	—	—	—	100	—	—	—	—	—
" trochoideum	—	—	100	—	—	—	—	—	—
Acanthostomella norvegica	—	—	—	100	—	—	—	—	—
Labora conica	80	—	100	—	—	—	—	—	—
" emergens	—	—	100	100	—	100	—	—	—
" reticulata	80	200	—	—	—	—	—	—	—
" strobila	400	400	—	—	—	100	—	—	—
Lohmanniella oviformis	—	—	—	200	100	200	—	—	—
" spiralis	—	—	—	—	—	—	—	—	—
Mesodinium rubrum	360	200	100	—	—	—	—	—	—
Woodania conicoides	120	—	—	—	—	—	—	—	—
Bodo marina?	520	1800	700	1600	100	800	400	100	400
Forms not identified	640	1000	1500	2000	—	600	600	100	200

atoms and *Ceratia* were the predominant forms and *Pontosphæra* was recorded only in comparatively small numbers; while in 1933 there was a predominant *Pontosphæra* vegetation. (The temperature of the surface layers was as high in 1932 as in 1933 and the salinity even higher.)

Gran (1933) recorded a poorer vegetation in the eastern part of the Gulf than further west. Since the observations were not taken in precisely the same locality, local differences in the plankton distribution may account for a part of the differences which have been recorded in the plankton of the two years; but we should hold it likely that yearly

TABLE III

Comparison of observations at stations 1722 and 1723

Station	Depth	Temp.	Salinity	O_2	Guinardia	Detonula	Pontosphæra
	meters	*° C.*	*‰*	*% sat.*	*cells/l.*	*cells/l.*	*cells/l.*
	1	13.17	32.07	107	—	200	146,000
	6	13.04	32.07	105	—	100	105,400
	12	12.52	32.09	108	—	—	198,000
	18	11.39	32.11	109	—	200	308,000
1722	30	10.46	32.19	107	—	100	130,200
	42	8.50	32.42	99	—	—	13,200
	54	7.01	32.63	94	—	200	9,500
	66	6.59	32.75	92	—	60	6,460
	78	5.77	32.80	88	—	—	1,880
	1	15.45	33.12	107	—	400	67,400
	5	15.47	33.15	112	100	600	50,500
	10	15.39	33.54	110	—	600	64,200
	15	13.92	33.27	111	700	1300	120,000
1723	25	8.67	32.51	97	1800	200	30,000
	30	8.47	32.53	96	5300	—	9,100
	40	8.44	32.56	94	3700	—	1,800
	50	8.21	32.56	93	3900	100	2,300
	60	8.07	32.60	94	2200	—	1,400
	70	7.98	32.60	94	3600	—	2,800

fluctuations in the composition of the phytoplankton are still more important in producing the state of affairs which has been observed.

For Georges Bank there is a similar difference in the quality of the phytoplankton. In 1932 there was observed here a rich *Rhizosolenia* vegetation with *Rh. alata* as the dominant species. This was then recorded in great numbers throughout the water column, with a maximum of 50,000 cells per liter at 25 meters. This species was also recorded in fairly large numbers from all the stations over the Bank, although it was not so abundant at the two outer stations. In 1933 at Station 1723 *Rhizosolenia alata* was recorded with a maximum number of

only 300 cells per liter. One might seek the explanation for the variation in the occurrence of this species in the fact that this station was located a little further out than the stations of the previous year. However, the abundance of *Guinardia flaccida* at Station 1723 seems to be a good indication that the station was located within the Bank area. The previous year *Guinardia* was also found in the deeper samples from all the stations over the Bank, while it was not observed in the samples from the deeper part of the Gulf (Gran, 1933, p. 177).

These few observations suggest that there exist considerable fluctuations in the composition of the phytoplankton of the waters of the Gulf of Maine and adjacent areas in the summers of different years, perhaps not so much in a purely qualitative respect as in the relative abundance of the various species. The observations on net haul material have given similar indications for the larger forms (Bigelow, 1926). These variations certainly are tied up with changes in the hydrographical conditions from one year to another. For example, the occurrence of *Guinardia flaccida* solely on the Bank (and only in the deeper layers) suggests dependence upon fairly turbulent waters. The question of the relationship between the phytoplankton production and the hydrographical conditions is, however, so complex that we shall not consider it further, since the data for only two stations would not suffice as a basis for discussion. At least two years of intensive simultaneous investigation of the hydrography, the plankton, and the various environmental factors would seem necessary in a study of the influence of the water movements on the supply and conditions of growth of algæ and an explanation of yearly fluctuations. A consideration of the relationship between phytoplankton production and hydrography, especially in regard to the seasonal changes in the phytoplankton occurring in the Gulf of Maine and the Bay of Fundy will appear shortly (Gran and Braarud, 1934).

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METHODS FOR THE DETERMINATION OF DISSOLVED ORGANIC CARBON AND NITROGEN IN SEA WATER

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Introduction

Certain problems in the nutrition of marine animals and in the general economy of organic life in the sea involve the determination of organic matter present in solution in the water. The methods worked out by the senior writer for the determination of dissolved organic matter in fresh waters are of no avail. In these methods (Krogh, 1930) the organic material is concentrated by evaporation of a large sample of the water and the organic material determined by combustion of the dry residue, but the sea water salts, present in quantities several thousand times as large as the organic material, make any attempt in this direction futile. All we can hope to accomplish by means of the resources at present available are determinations—more or less approximate—of the nitrogen and carbon present in organic combination, and these simplified analytical problems are what we have endeavored to solve.

Assuming according to the scanty and not especially convincing estimates to be found in the literature (Atkins, 1922; Brandt, 1916–20; Gran and Rund, 1926; Harvey, 1925; Natterer, 1892–1900; Vernon, 1898) that we should have to deal with quantities of at most a few milligrams per liter of water, it has been our endeavor to work out microchemical methods of handling water samples of small volume. Large samples are in many cases not available, and, more important, the necessary time and expense in preparing large samples for analysis would be a serious drawback.

The Determination of Organic Nitrogen

A large number of trials were made with micro Kjeldahl methods, but they all failed. The quantity of sulphuric acid necessary for the Kjeldahl treatment becomes relatively very large on account of the salts, and it was not found possible to obtain or prepare sulphuric acid of sufficient purity. All samples examined would, after the Kjeldahl

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heating in ammonia-free air, give off a variable amount of ammonia when distilled after addition of excess sodium hydroxide. The sulphuric acid was treated in various ways to get rid of this "organic" nitrogen and in the final procedure evaporated in a current of oxygen. The mixture of vapor and oxygen was taken through a quartz tube containing platinum heated along a length of about 10 cm. to 800–900° C. and along a further length to about 400° C. From the distillate supposed to contain some NH_3 a portion was again distilled off in a high

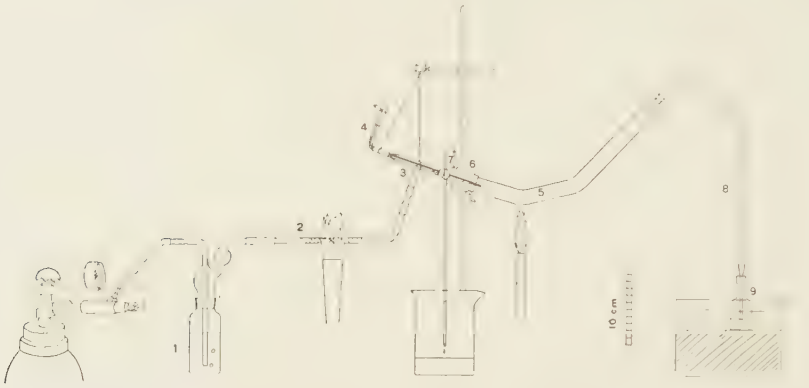

Fig. 1. Apparatus for the determination of dissolved organic nitrogen in sea water.

(1) Sulphuric acid wash bottle.
(2) Quartz reduction tube.
(3) Glass connecting head.
(4) Tube for introduction of sea water sample.
(5) Silver tube.
(6) Water jacket.
(7) Cooling water outlet.
(8) Silver condensing tube.
(9) Receiver.

vacuum and tested, but even with this acid reliable results could not be obtained, because the acid itself would in heating yield variable amounts of nitrogen of the order of 0.5 to 2 γ (0.0005 to 0.002 mg.) N per milliliter.

The old Will-Warrentrapp method was suggested to us by Professor Biilmann and we finally succeeded in working out a modification of it suitable to our needs.

In this method the organic material is heated to about 500° C. with alkali in an atmosphere of hydrogen and, in the absence of oxygen, most of the organic nitrogen is liberated as NH_3, while a small part may be driven off as amines. No type of glass and not even quartz will stand this treatment more than a few times and we therefore do the combus-

tion in a silver tube. The ammonia (+ amine) is taken up in N/100 HBr, combined with sodium hyprobromite, and the excess hyprobromite titrated according to the method of Teorell described in detail by Krogh in the preceding paper in this journal.

The apparatus is shown in Fig. 1. Hydrogen from a cylinder is bubbled through sulphuric acid (1) to remove any traces of NH_3 and to indicate the rate of flow which should be of the order of 10 to 20 ml. per minute.

The purification of the hydrogen before it enters the silver combustion tube may be accomplished in several ways. The simplest arrangement is to bubble it through sulphuric acid to remove any traces of NH_3 and then to pass it through a quartz tube (2) with metallic copper as a contact substance heated to a dull red heat to remove traces of oxygen. The frequent presence of arsenic and antimony traces in the copper, however, require caution in its use. A safer procedure is to bubble the gas through sulphuric acid, then through a long absorption train containing pyrogallic acid, again through sulphuric acid, and finally to pass it through the quartz tube over metallic platinum foil heated to a red heat.[2]

The connecting piece (3) is glass. The solution for combustion is added from a pipette through (4). A small part of the silver combustion tube (5) is water-jacketed and the clamp (6) grips the water jacket (7), which is a short piece of brass tubing. The current of water is regulated so that the water in the jacket will just boil when the maximum heat is applied to the combustion tube.

An analysis is performed as follows. A pellet of sodium hydroxide is placed in the combustion tube (5) which is then connected up to (3) and the system is washed out with hydrogen. The water sample—usually 5 ml.—is run in through (4). A collecting bottle with 1 ml. of N/100 HBr is placed in a basin with cold water so that the narrow tip of the silver tube will just dip into the acid. The combustion tube is heated gently at first to boil off the water and finally ignited to a very dull red heat which can be observed only when the room is dark. When sea water is analysed some ammonia will become occluded in the salts, and it is therefore necessary, after cooling, to run in an additional 2 ml. of "blank" water which should contain no nitrogen, or at all events only a known small amount, and evaporate this also. Finally the whole tube including (8) is heated (below the incineration point) by moving the flame so as to bring every trace of water into the collecting bottle (9). We have found that two determinations can be made in series without

[2] We are indebted to Dr. Homer P. Smith for having worked out this second procedure.

opening the combustion tube, but thereupon the salts must be washed out. To do this (3) is disconnected, a little nitrogen-free water poured in and heated to near boiling. The water jacket is emptied. The tube is turned slightly up and down in the clamp and the water poured out. This is repeated once. A fresh pellet of sodium hydroxide is put in and after connecting up, 2 ml. of N-free water is added. When this has been distilled off and the tube ignited it is ready for the next two determinations.

The most serious difficulty in this technique is the preparation of sufficiently pure water for the washing operations. In Copenhagen water from the municipal supply could be distilled off first from an alkaline solution of permanganate and again from sulphuric acid and permanganate. In Woods Hole and in Cambridge, Massachusetts, acceptable water was prepared by distillation from strongly alkaline permanganate and then from very dilute phosphoric acid. Both distillations were carried out in a current of washed air in all-Pyrex stills.

The distillate is tested both for free ammonia and for "organic" nitrogen by the combustion method. One sample of water gave the following results:

Test for free ammonia—Titration of 1 ml. NaBrO with

2.5 ml. water directly,	1.89_5	Mean = 1.89 ml. Napthyl red
5.0 ml. water directly,	1.89_5	
7.5 ml. water directly,	1.89	
2.5 ml. water + 2.25 γ N,	0.66	

Hence 1 ml. napthyl red $= \frac{2.25}{1.89-0.66} = 1.83\,\gamma$ N.

Test for "organic" N—Titration of 1 ml. NaBrO with

2.5 ml. water "burnt,"	1.85,	equivalent to 0.04 ml. napthyl red,
5.0 ml. water "burnt,"	1.80,	equivalent to 0.09 ml. napthyl red,
7.5 ml. water "burnt,"	1.77,	equivalent to 0.12 ml. napthyl red,
15 ml. water "burnt,"		0.25 ml. napthyl red.

Hence 1 ml. of the "blank" water contains 0.030 γ N (0.030 mg. per liter). The single determinations show that within limits of error of the titration the nitrogen obtained as ammonia is proportional to the quantity of redistilled water used.

When 2 ml. of "blank" water are used in a combustion of sea water they necessitate a correction of 0.06 nitrogen or 0.03 ml. napthyl red to be subtracted from the value obtained. Distillation of deep sea water gave a satisfactory result, *i.e.*, a low and constant "blank" value.

A series of determinations on the same sea water taken in varied quantities gave the following results:

Blank value	1.90		1.88	ml. napthyl red	
2.25 γ N	0.65_5		0.67		
	1.24_5		1.21	1 ml. napthyl red = 1.83 γ N	
					Sum
Vol. of sea water, ml.	5.25	5.1	3.0	7.0	20.35
ml. napthyl red used (corrected for NH_3 in "blank" water)	0.73	0.72	0.47	1.005	2.925
γN in 10 cc.	2.54	2.58	2.86	2.62	2.64, or 0.264 mg./1.

From this quantity the amount present as preformed NH_3 must be subtracted to arrive at a figure for "organic" nitrogen.

Some control determinations on dilute solutions of glycine, creatine, and urea averaged about 5 per cent too high, but the correction for the organic matter in the distilled water used was somewhat uncertain. Analyses on acetamide and cocaine hydrochloride solutions also proved the efficiency of the method. Nitrates do not affect the results in the least. Table I, below, summarizes the results from a number of control experiments. In addition some results on various distilled waters are given.

TABLE I

Control analyses for organic nitrogen. Concentrations are in γ of nitrogen per liter (mg. per cubic meter).

Cocaine solutions		Nitrate solutions		Ordinary laboratory dist. H_2O	Conductivity water
Known	Found	Known	Found		
800	792	250	0	90	44
"	820	"	10	94	58
"	836	"	−6	85	40
"	798	"	14	87	46
"	778	"	4		
"	816	"	12		
400	410				
"	400				
"	388				

THE DETERMINATION OF ORGANIC CARBON

We have worked out a wet combustion method not very different in principle from that described by Lieb and Krainick (1931).

The water sample is acidified to drive out preformed CO_2. Most of the chlorine is precipitated out with thallium sulphate and the water thereupon evaporated to dryness. The dry residue is oxidized with

chromic and sulphuric acids in a current of carefully washed air which carries the evolved CO_2 and CO through an oxidizing combustion tube and then into a baryta solution which ultimately is titrated with HCl.

The necessary reagents are prepared as follows:

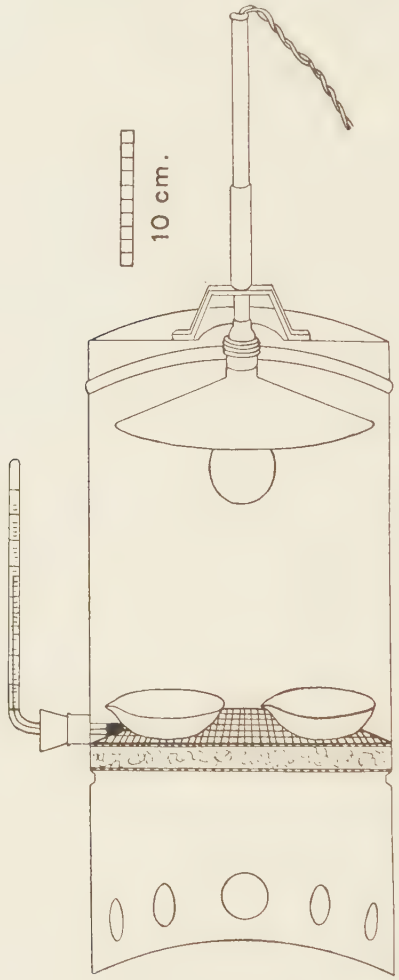

FIG. 2. Radiation evaporation chamber.

(1) Phosphoric acid dissolved in pure redistilled water and diluted to about n/10.

(2) Methyl red used as indicator.

(3) Thallium sulphate (Tl_2SO_4). The purest preparation available is melted in a crucible and stirred well so as to remove by combustion

any trace of organic material. We use for stirring a glass tube connected with an oxygen cylinder, but this may not be necessary. After cooling, the salt is carefully powdered and preserved for use in a wide-necked glass-stoppered bottle.[3]

(4) Chromate-sulphuric acid for oxidation: 10 grams potassium bichromate, 4 grams silver chromate, and 1 gram chromic acid are mixed in a crucible and melted together. The resulting solid block is pulverized.

To 50 cc. redistilled water and 50 cc. of the purest sulphuric acid we add 6 grams of this chromate mixture and 3 grams cerium sulphate. The mixture is put into the storage bottle (Fig. 3), placed in a glycerine

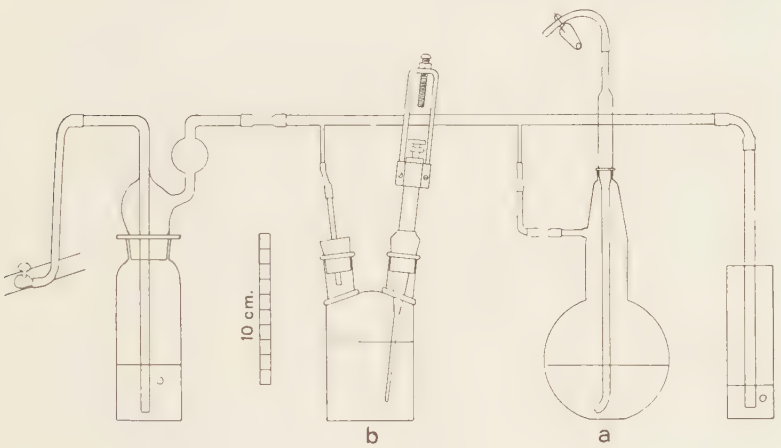

Fig. 3. Arrangement for storing reagents.
(a) Ceric-chromic sulphuric acid.
(b) Baryta solution and syringe-pipette.

bath at 125°–130°, and heated under aeration with CO_2-free air for about 24 hours. The mixture is kept protected against CO_2, as shown in Fig. 3 (*a*).

(5) Potassium bichromate c.p. kept as a fine powder in a glass-stoppered bottle.

[3] The thallium chloride precipitate from the analyses can be recovered as thallous sulphate easily. The precipitate is gathered into a large round-bottomed centrifuge tube in which it is washed and centrifuged several times, minimal amounts of cold distilled water being used for each washing. The washed precipitate is placed in a casserole with a 50 per cent excess of pure sulphuric acid and the whole cooked at 400° C. for 24 hours in a hood. The casserole should be covered with a cover propped in place so as to give a free clearance of about an eighth of an inch all around. After this digestion the temperature is raised to boil off all the sulphuric acid (450° C.) for about 6 hours. After cooling in a desiccator, the solid mass of thallous sulphate is broken out and carefully pulverized in a mortar. It is now ready for use.

(6) Hydrochloric acid N/3 used for titration in a Rehberg microburette. One cubic millimeter of this acid corresponds to 2 γ carbon. The stock of acid should be kept in a bottle provided with a ground cap over the stopper to insure against evaporation, which will often take place to a quite appreciable extent from ordinary glass-stoppered bottles.

(7) Baryta solution about N/12 to N/15 prepared with 0.5 per cent $BaCl_2$ (and cresolphthalein[4]). The baryta solution is kept in a bottle protected as shown in Fig. 3 (*b*) and with a 0.5 ml. syringe-pipette mounted on top. The volume of baryta delivered by the pipette is regulated so as to correspond to between 90 and 100 mm.^{3} of the n/3 acid.

The apparatus and procedure is as follows: The salinity of the water is determined. (A hydrometer will give sufficient accuracy and all samples of ocean water can be taken as having a salinity of 35‰.) From 10 to 25 cc. of the sea water are measured off in a white porcelain basin, methyl red is added, and standard phosphoric acid is run in from a 2 cc. burette or measuring pipette until a persistent red color is obtained. The corresponding volume of H_3PO_4 N/10 is added to the samples to be analyzed. These samples (we use 25 ml. each) are measured off in centrifuge bottles as shown (Fig. 4, *c*) and thallium sulphate is added. The quantity of Tl_2SO_4 necessary depends upon the salinity. We allow an excess of about 30 per cent, which means that for a 25 ml. sample of ordinary sea water 2.5 grams of pure thallous sulphate is sufficient; a greater excess is not harmful.

The vessels are shaken in a suitable shaking machine for 10 or more hours. The shaking need not be violent. Good results have been obtained by putting in two glass balls and placing the bottles on a slowly rotating vertical wheel. After the shaking the bottles are centrifuged for a few minutes and 20 ml. of the supernatant fluid can now be pipetted off for the analysis.

These samples are evaporated down to dryness in porcelain basins. Several methods have been tried. With evaporation in an oven at 105°, splashing is difficult to control and there is some danger of losing organic matter. Evaporation in a vacuum oven is a slow process and some splashing generally occurs. The simple radiating oven shown in Fig. 2 has been found very useful. It is made from tinplate, the surface of which reflects much light and heat. A rapid current of air is maintained by the heat of the 60-watt lamp, and as the air is filtered through the layer of cotton wool, contamination with dust is effectively guarded against. The temperature may rise to 70° C., but not until the samples are almost dry, which takes about 4 hours. Eight hours will ensure

[4] Enough cresolphthalein is added to give a deep red color in the bottle and to give a pale but very distinct red when placed in a tube of 6-mm. bore.

thorough desiccation. If it is necessary to leave the samples for some time after drying, they should be kept well protected against moisture and dust—preferably in a desiccator.

The white crust of salts should be fairly easy to scrape off. This is transferred with great care to a combustion flask with side arm. We put in a couple of glass balls and one spoonful (about 0.2 gram) of potassium bichromate. The combustion flasks are cleaned with thiosulphate solution, then with bichromate cleaning fluid, and finally are

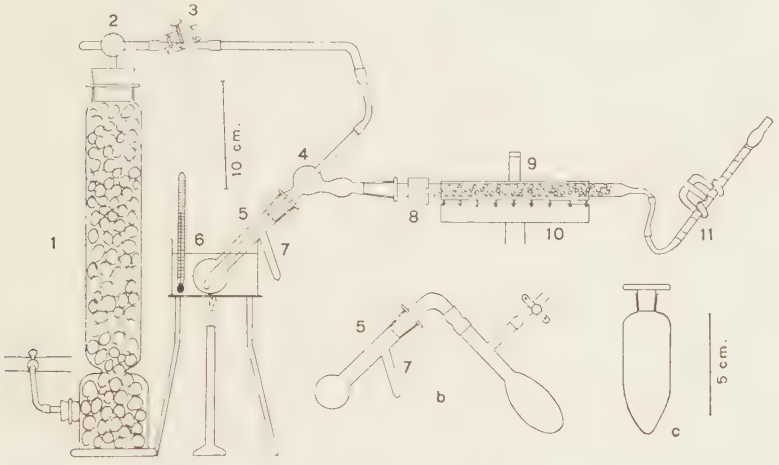

Fig. 4. Combustion apparatus for the determination of dissolved organic carbon in sea water.

(1) Soda lime tower.
(2) Three-way tap.
(3) Pinchcock air-flow regulator.
(4) Connecting head.
(5) Combustion flask.
(6) Glycerine bath.
(7) Side arm to hold oxidizing reagent.
(8) Pyrex combustion tube.
(9) Tube for thermometer in metal jacket.
(10) Long burner.
(11) Baryta tube.
(b) Arrangement for drying flasks rapidly.
(c) Stoppered centrifuge tube.

rinsed just before use with a little redistilled water which is poured out as completely as possible and dried. This is done quickly by immersion in hot water after connection with a filter pump (Fig. 4, *b*).

When the combustion material and bichromate have been thoroughly mixed, the suitable volume of chromate-acid is put into the side arm (*7*, Fig. 4). For a sample of ocean water 3 ml. are necessary. The standard ground joint is lubricated by just touching it with the tip of the pipette delivering the chromate acid.

The flask is connected with the combustion apparatus shown in Fig. 4, and the beaker with glycerine (6) used as a bath is put in position. Air at a low pressure (*e.g.* from a reversed filter pump acting as a blower) passes through moist soda lime in (1) to remove CO_2 and sweeps through the entire system.

The combustion tube (9 in Fig. 4) is arranged to remove completely all last traces of halogens or halogen acids and to ensure that any CO will be converted to CO_2. In traversing it the air passes, successively, asbestos shreds, lead chromate dispersed in asbestos, cupric oxide, asbestos, platinum foil, asbestos impregnated with finely divided metallic silver, and finally asbestos shreds. The combustion tube is made from Pyrex or other hard glass. A length of 14 cm. is surrounded by a copper tube (9) heated by the burner (10). A thermometer can be placed in (9) and the burner regulated once for all so as to give a temperature between 400 and 450° C., which is maintained throughout when analyses are in progress.

At first a fairly rapid current is maintained to wash out the system for about 10 minutes. The flow of air is regulated by the clip (3) and may be observed in the absorption tube (11), which at this stage contains a single drop of water. During this period the glycerine bath is slowly warming.

When the temperature in the glycerine bath has reached about 80–90° C., the air current is reduced so far that baryta solution can be put into the absorber (11) without danger of its being spilled out. The combustion flask is now turned so as to transfer a little of the chromate-acid in the side arm to the bulb. This procedure must be carried out with some caution because it lowers the temperature and absorbs any water vapor present and is therefore liable to suck back baryta from (11) into (8), which of course will spoil the analysis. When all the chromate-acid has been transferred it is often necessary to mix the contents in the bulb by several times revolving the tube (5). When a temperature of 120° is reached the combustion is continued for 30 minutes, which will be sufficient to drive off all the CO_2 formed.

A clip is now placed on the rubber tube of the absorber, which is transferred to a Rehberg micro-burette stand and connected with a slow current of CO_2-free air. During the titration the tip of the micro-burette dips about halfway down in the tube and the baryta is kept moving up by means of the air current. When the end point is almost reached the absorber is removed from the burette, the solution is rinsed about in the narrow portion of the absorber and afterwards in the bulb, the absorber is replaced, and the titration is finished. The end point can be judged with an accuracy of 0.1 mm.[3]

Blank determinations on the chromate-acid alone and with thallium sulphate are necessary. The chromate-acid will always give off a certain amount of CO_2 corresponding to 2–10 γ carbon, and since this liberation depends to some extent on time and temperature, it is essential that the blanks should be treated in every way like the analysis proper. The average of several blank titrations should be used for the determinations.

Example: baryta titrated directly 93.0, 93.0 mm.^{3} N/3 HCl. Blanks with 3 ml. CrA 88.1, 87.8, 89.5, 87.4, 89.3, 89.3 = 88.6 average. Samples of sea water, 25 meters, samples 1–2 untreated, 2–4 sterilized with several drops of $NaHSO_3$. The untreated samples require 0.50 ml. of H_3PO_4 N/10, the others are already acid. The same amount of Tl_2SO_4 added to each.

Titrations, mm.^{3} N/3 HCl:

Untreated 67.0, 63.1; sterilized 64.4, 63.6.

Average 64.5; blank 88.6.

Carbon found $2 \times 24.1\,\gamma$ in 20 ml. = 2.41 mg./liter.

The accuracy to be obtained does not exceed 0.1 mg./liter. When, as in Copenhagen, there is much coal dust in the air, it is essential that the combustion apparatus be kept closed except during the short intervals of time when combustion flasks are changed.

A recent series of blanks with the new mixture, containing cerium sulphate, but prepared from ordinary pure sulphuric acid, gave the following values for 0.5 ml. chromate-acid: 0.7, 1.2, 1.0, 0.7, corresponding to 3.6 γ C per ml. These low blank values indicate that the preparation of special sulphuric acid may not be necessary.

Organic Matter in Colloidal and in True Solution

The problem of the amount, if any, of organic material in sea water present in the colloidal state is of considerable importance. It is to be expected that the disintegration of organisms will yield a considerable proportion of colloidal material, but whether this will remain in the colloidal state for any length of time is at present unknown. The nitrogen determinations described above will comprise everything present, whether suspended, colloidal, or dissolved, but the precipitation of chlorides, which is a necessary initial step in the carbon determinations, will throw down all suspended particles and may be expected to remove also a variable proportion of colloids.

Colloids can be removed from water samples by ultra-filtration, but care must be exercised to guard against contamination from the filters and the filtering apparatus. We have used ultrafilters from "Mem-

branfilter Gesellschaft," Göttingen, Germany, designated as 70 minutes, which means that at a pressure difference of 1 atm. 100 ml. of pure water will be filtered through 100 sq. cm. in 70 minutes.

Our filtering apparatus is a modification of the Thiessen Ultrafiltration apparatus and it will stand a pressure of about 5 atm. The filtering surface of 4 $cm.^2$ will therefore allow the filtration of about 15 ml./hour. We generally pass the water first through a 25–35 sec. filter which will remove all suspended matter including bacteria.

If the water contains more than a trace of colloids the rate of filtration through a 70-minute filter at a constant pressure will gradually become reduced, but in the few experiments so far made with ocean water no decrease in rate was observed and no difference could be found between carbon or nitrogen values before and after ultra-filtration.

The detailed results of the application of these methods to sea water collected from various localities and depths will be presented in separate papers.

Summary

A method for the determination of organic nitrogen in solution in sea water is described in detail. The principle of Will-Warrentrap, involving digestion at 500° C. with caustic soda in an atmosphere of hydrogen, is used. The sensitivity of the method is roughly 0.0003 mg. N, giving an accuracy in sea water of about 5 per cent in 5- or 10-ml. samples.

A wet combustion method for the analytical determination of dissolved organic carbon in sea water is presented. Halides are removed with thallous sulphate, the evaporated sea water residue is oxidized by means of ceric-chromic sulphuric acid, and the evolved carbon dioxide eventually determined by acid titration of baryta solution through which the gases have passed. The accuracy approaches 0.1 mg. carbon per liter.

Differentiation between organic matter in colloidal and in true solution is made by analyses before and after ultra-filtration, using "Membranfilter Gesellschaft" collodion filters and a modification of the Thiessen ultrafiltration apparatus.

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FURTHER OBSERVATIONS ON THE DIURNAL MIGRATION OF COPEPODS IN THE GULF OF MAINE

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The following observations were undertaken to obtain further information on the diurnal migration of plankton in relation to environmental conditions. It was felt that the influence of temperature should be more carefully tested, particularly at the thermocline. The possibility that diurnal migration might be caused or controlled by reactions to food organisms required investigation. More precise and more extensive observations were desired on the effect of the changes in submarine irradiation with depth and with time of day. The observations were made at two stations in the Gulf of Maine, the first (Station 1722) occupied for 54 hours on July 14–16, 1933, in the deep central part of the Gulf but farther off shore than the stations of the previous year (Clarke, 1933), and the second (Station 1723) occupied for 24 hours on July 17–18, 1933, on the eastern edge of Georges Bank. By comparing the behavior of the copepods at these different localities, it was believed that the importance of differences both in environmental factors and in physiological condition of the animals would be revealed.

Methods

The same methods were used for studying the migration as before with the following modifications. The nets were provided with new brass buckets in which the monel metal netting was coarser (18 strands/cm.), thus allowing faster drainage and minimizing clogging. The strap (15, Fig. 1*A*, Clarke, 1933) used to hold the tail of each net at the edge of its frame preliminary to lowering was replaced by a light brass chain through an appropriate link in which the pin (14) was inserted. The pursing rope (*P*) and the cord (18) were provided with spring clips for fastening them to the upper clamp (2) and the handline (19) respectively, thus saving the time formerly consumed in tying knots. To aid in keeping the ship in the same body of water throughout the whole period of observation, a buoy was constructed consisting of a keg with a pole thrust through it at right angles to its long axis. Since our intention was to study the plankton in the upper 50 meters

[1] Contribution No. 48.

of water particularly, a sea anchor was attached below the keg at a depth of 25 meters. The buoy therefore tended to stay with the body of water at that average depth, and since the keg floated three-quarters submerged, it had very little windage. The buoy was equipped with a flag during the day and with two hurricane lanterns at night and could be seen easily for several miles. The depth of the hauls is indicated in the tables. At the deep station the copepods migrated to such a depth that three series of hauls were sometimes necessary. The qualitative and quantitative analysis of the catch and the length measurements were very kindly carried out for me by Mr. Charles Wheeler assisted by Mr. David Lillie.

General Vertical Distribution

The catches were composed almost entirely of *Calanus finmarchicus* (Gunner). A small number of *Metridia lucens* (Boeck)—mostly adult females—was taken also in some cases (Tables I and II). At the deep station *Calanus* copepodid stage V was the most numerous group present. This group, together with copepodid stage IV and the adults of both sexes, were represented at some time of the day at every depth down to 174 meters. Very few individuals in copepodid stage III occurred at this locality. *Metridia* was generally confined to the deeper levels, although at night the range of the adult females and the immature did extend upwards to 6 meters. Since the water was 260 meters deep at this station, there was a considerable depth below the lowest haul which was not sampled at all. At the Bank station the adult females and copepodid stage V of *Calanus* were the most numerous group, but the adult males and stages IV and III were also well represented. All the *Calanus* groups were found at all levels. *Metridia* occurred chiefly at the deeper levels. Since bottom was at 65 to 75 meters at this station, the whole depth of water was sampled in this case.

To aid in distinguishing seasonal broods the cephalothorax length of copepods in the various sex and age groups were measured from representative hauls made at different depths and at different hours during the two stations (Table III). From Table IV it is seen that the ratio of the total length to the cephalothorax length of *Calanus* is extraordinarily constant. This means that relative shape remains the same in spite of differences in size.

The cephalothorax length of *Calanus* adult male changes very little with depth or with time of day. In the case of the adult females considerable constancy of length exists for all the hauls within each station, but at the deep station the averages range from 2.654 mm. to 2.722 mm.,

whereas at the Bank station the range is from 2.536 mm. to 2.674 mm. If this difference be regarded as significant, it may indicate that we are dealing with two different broods (*cf.* Russell, 1928, 1934; Marshall, 1933). None of the averages for the adult females is as low as that found for the 6-meter level previously, namely, 2.445 mm. (Clarke, 1933, Table VI). Furthermore, in the present case the range is not as great and there is not a distinct increase in length with depth. The average lengths of *Calanus* copepodid stage V show a little more variation at each station, but there is no consistent change with depth and no clear-cut difference between day and night hauls. The range of lengths is about the same at the two stations, and the average size is slightly greater than that found the previous year. Average lengths for copepodid stage IV range from 1.853 mm. to 1.903 mm. at the deep station and from 1.743 mm. to 1.867 mm. at the Bank station. The animals taken this season were generally larger than those of the same stage taken previously. The lengths of 25 specimens of *Calanus* copepodid stage III (Haul 420) ranged from 1.275 to 1.488 mm. and the average, 1.403 mm., is greater than that found the year before. For *Metridia lucens* the range for 27 specimens of the adult females from Haul 342 is 1.828 to 2.253 mm. and the average, 2.014 mm., falls within the range reported previously. In general the length measurements of all the copepods vary much less than was the case the year before and there are no regular changes within each group.

Environmental Conditions

The intensity of light present at different depths and its changes during the course of the day were measured at both the stations (Clarke and Oster, 1934). For the sections of the spectrum considered, red light had very nearly the same intensity as blue at the surface of the water but its intensity diminished very much more rapidly with depth. The intensity of red light was reduced to 1 per cent of its value at the surface at a depth of 11 to 12 meters whereas blue light did not suffer a corresponding reduction until a depth of 26 to 40 meters was reached. Since the copepods appear to have migrated downwards more than 100 meters at the deep station, it seems probable that in so far as their movement is a response to light, it is the blue component rather than the red which is chiefly concerned. Lines of equal intensity for blue light only have been represented in all the figures of diurnal migration.

At the deep station the temperature of the water was measured every 5 meters to a depth of 50 meters and the series was repeated 19 times

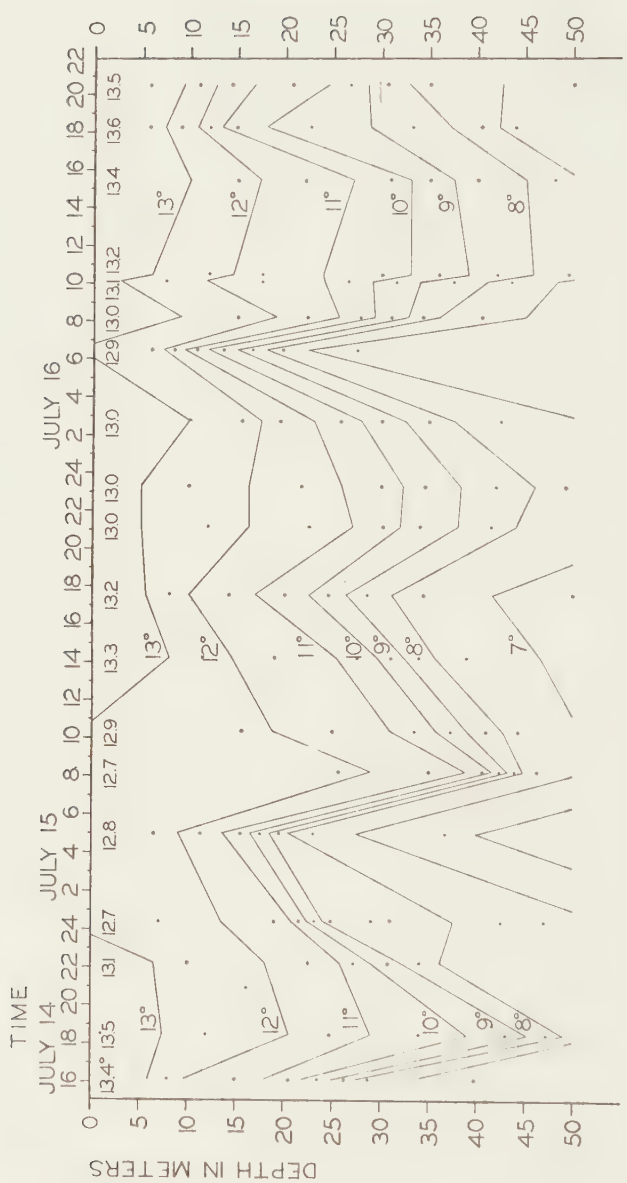

FIG. 1. Variation in temperature down to 50 meters at the deep station (Station 1722). Nineteen series of measurements were made at the hours indicated. The black dots indicate the half-degree points. The temperatures at the top are for the 1-meter level.

during the 54-hour period[2] (see Fig. 1). The isotherms show two sharp peaks: one at 5 o'clock, July 15, and the other at 6 o'clock, July 16. There are three less prominent peaks alternating with these.[3] The diurnal heating effect of the sun can be seen in the variation of the temperature at 1 meter. The difference between the highest and lowest temperatures at this depth was only 0.9° C. At a depth of 28 meters a change of 5° C. occurred in the 3 hours between 5 and 8 o'clock on July 15. Since it would be impossible for a large body of water to change its temperature so quickly, the effect must have been produced by a vertical movement of whole strata of water. This is substantiated by the fact that the thermocline is seen to have been periodically displaced. For example, during the 3 hours following 5 o'clock on July 15 it is moved downwards 25 meters. The temperatures below 50 meters at the deep station (Table I) were measured at three different times and found to vary on the average by about half a degree. For the one temperature observation taken at the Bank station the thermocline occurred between 15 and 20 meters. Data showing variation with depth of oxygen, phosphate, nitrate, and salinity for these stations are given by Braarud (1934, Tables I and II).

The vertical distribution of the phytoplankton at both stations has been reported by Braarud (1934). The phytoplankton was generally poor in both localities, but coccolithophorides, peridinians and ciliates were found above the thermocline and diatoms existed below it. At the deep station *Pontosphæra Huxleyi* was the dominant form and occurred in maximum numbers at 18 meters. Although this species was also the most numerous at the Bank station (maximum abundance at 15 meters), the dominant form in respect to bulk was the much larger *Guinardia flaccida* with a level of maximum abundance at 30 meters.

Diurnal Migration

The sex and age groups of *Calanus finmarchicus* all exhibited much the same type of diurnal migration at the deep station as may be seen from Figs. 2 and 3. The copepods of all groups were distributed chiefly below 80 meters at 1730 (Local sun time) on July 14. At 1930 there was an indication of an upward movement, but at 2130 maximum numbers occurred at the bottom of the series. Probably a rapid upward migration took place during the interval between these two series,

[2] In connection with meteorological investigations of Prof. C. G. Rossby and Mr. R. T. Montgomery.

[3] Similar fluctuations in temperature were observed by Prof. C. G. Rossby and Prof. A. C. Redfield on June 1, 1934 from the "Atlantis" while anchored at Station 2212 (not yet reported).

with the result that at 2130 the major part of the migrating population was present above 70 meters where no hauls were made. Support for this suggestion is furnished by the fact that copepods occurred in numbers in the upper strata at 20 o'clock on the following two evenings. Furthermore, since a large population existed near the surface at dawn on July 15, an upward migration must have taken place at some time during the night. The descent the following morning was very rapid, for at 0530 the maximum occurred at 66 meters or below and at 9 o'clock the entire population had sunk below 100 meters. At 1630 the upward movement had begun and proceeded rapidly until about 20 o'clock. Not much change in distribution took place during the remainder of the night, but in every case except that of the adult females, the population occurred at a slightly lower level before dawn the next morning (*cf.* Worthington, 1931; Nicholls, 1933). The migration on July 16 followed very closely the course run on the previous day. The indication of a concentration of adult males at the 6-meter level at 0430 on July 16 is probably due to a sampling error, the great majority of animals undoubtedly being below 54 meters at that time (*cf.* Table I). The general nature of the migration of *Calanus* at this station is similar to that observed by Nicholls (1933) for the adult females in the Clyde Sea-Area.

The slight differences in the behavior of the sex and age groups are chiefly matters of the distance through which the population moved. Thus, although all groups migrated to a depth of 120 meters or more during the day time, the adults descended farther than the immature on July 15. At night, the range of every group extended up to 6 meters but the level of maximum abundance was never higher than 42 meters for the males and never higher than 30 meters for copepodid stages V and IV, whereas for the females it occurred at 6 meters in the first series on July 16. Generally throughout the day the males were distributed deeper than the other groups.

A consistent though rough correlation between the vertical distribution of the copepods and the intensity of the submarine irradiation is apparent from the figures. Practically no individuals were taken from zones where the light intensity was 100 μw or greater. Early in the morning the center of the population coincides fairly closely with the 1.0 μw line, but in the next few hours the copepods descend more rapidly than the lines of equal intensity do, until at 9 o'clock maximum numbers occur at depths where the irradiation is less than 0.01 μw. It appears that insofar as the animals are reacting to light a higher intensity is required to initiate the migration than is required to cause it to continue. At 16 o'clock and thereafter the copepods were found in strata where

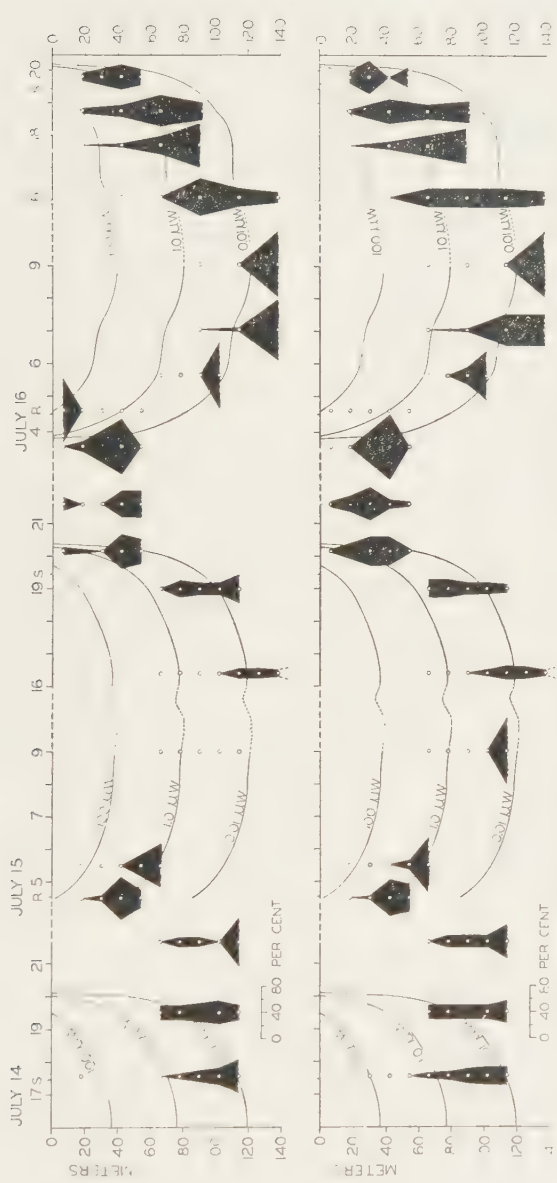

FIG. 2. Upper—*Calanus finmarchicus*, adult male. Lower—*Calanus finmarchicus*, copepodid Stage IV.

The diurnal migration during the 54-hour period at the deep station (Station 1722). The black circles and white dots indicate the depths at which hauls were made. The changes in the intensity of the blue component of daylight are shown by the lines representing the depths at which 100 microwatts/cm.^{2}, 1.0 microwatts/cm.^{2}, etc. occurred. Where the lines are broken, a shortening of the time scale is indicated merely; observations were not discontinued during these periods. S = Sunset. R = Sunrise.

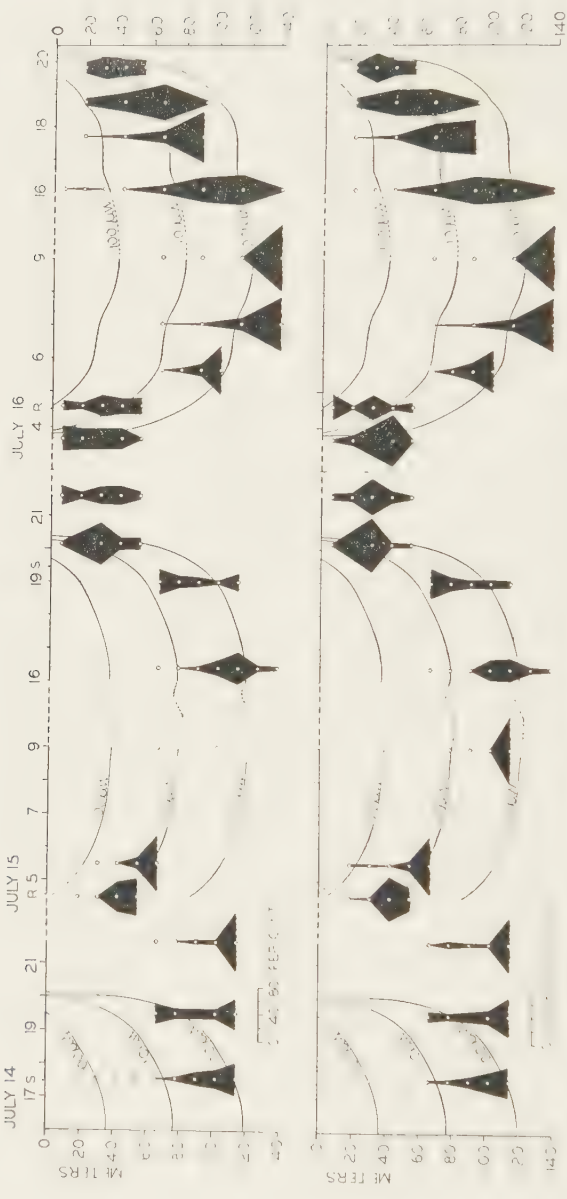

FIG. 3. Upper—*Calanus finmarchicus*, adult female. Lower—*Calanus finmarchicus*, copepodid stage V. The diurnal migration at the deep station (Station 1722).

the irradiation was more than 0.01 μw and in some cases even more than 1.0 μw. This tolerance of higher irradiation in the latter half of the day can be explained as the result of decreased sensitivity following light adaptation (*cf.* Worthington, 1931; Russell, 1931). *Calanus* copepodid stage III was almost entirely absent at the deep station.

Males, females, and immature of *Metridia lucens* occurred in the deep hauls and in the shallower hauls made at night. Toward the end of the afternoon on July 15 *Metridia* was encountered from 138 to 174 meters, and on July 16 from 90 to 138 meters. Females and immature reached the 6-meter level and males reached the 42-meter level at 2138 on July 15, but these strata were deserted early in the morning.

The observations made on the succeeding days at the Bank station revealed an entirely different vertical distribution and diurnal migration of *Calanus,* the major portion of the population being confined to the upper strata at all times (*cf.* Nicholls, 1933, p. 155; Russell, 1934). This station was about 100 miles south of the preceding and located in a physiographically distinct part of the Gulf, but, as described above, the environmental conditions were not profoundly different. The sex and age groups of *Calanus* all exhibited the same general behavior (Table II and Figs. 4 to 6). At 16 o'clock on July 17 maximum numbers occurred at depths of from 5 meters to 25 meters, and as night came on there was a slight movement upwards. At 3 o'clock the next morning most groups were rather evenly distributed in the upper 30 meters but an hour later there was indication of a concentration toward the surface again (*cf.* Russell, 1926). Soon after 6 o'clock the main downward migration began. The upper strata were progressively deserted during the remainder of the morning until at 14 o'clock maximum abundance occurred at a depth of 25 meters. By 1530 the copepods had started to move toward the surface again.

The adult males (Fig. 4, upper) did not migrate toward the surface as strongly as did the other groups of *Calanus.* The males were rather evenly distributed throughout the day and the maximum was usually to be found at 12 to 15 meters. The adult females (Fig. 4, lower) not only were much more abundant in the uppermost water layer but also were taken in even smaller numbers below 30 meters. This group exhibited little tendency toward uniform distribution during the night and did not leave the surface as early in the morning as did the males. The copepodid stage V population (Fig. 5, upper) was not highly concentrated, being well represented at all depths even down to 65 meters, but its vertical movement was slightly more extensive than that of the adults. Copepodid stage IV (Fig. 5, lower) migrated more strongly toward the

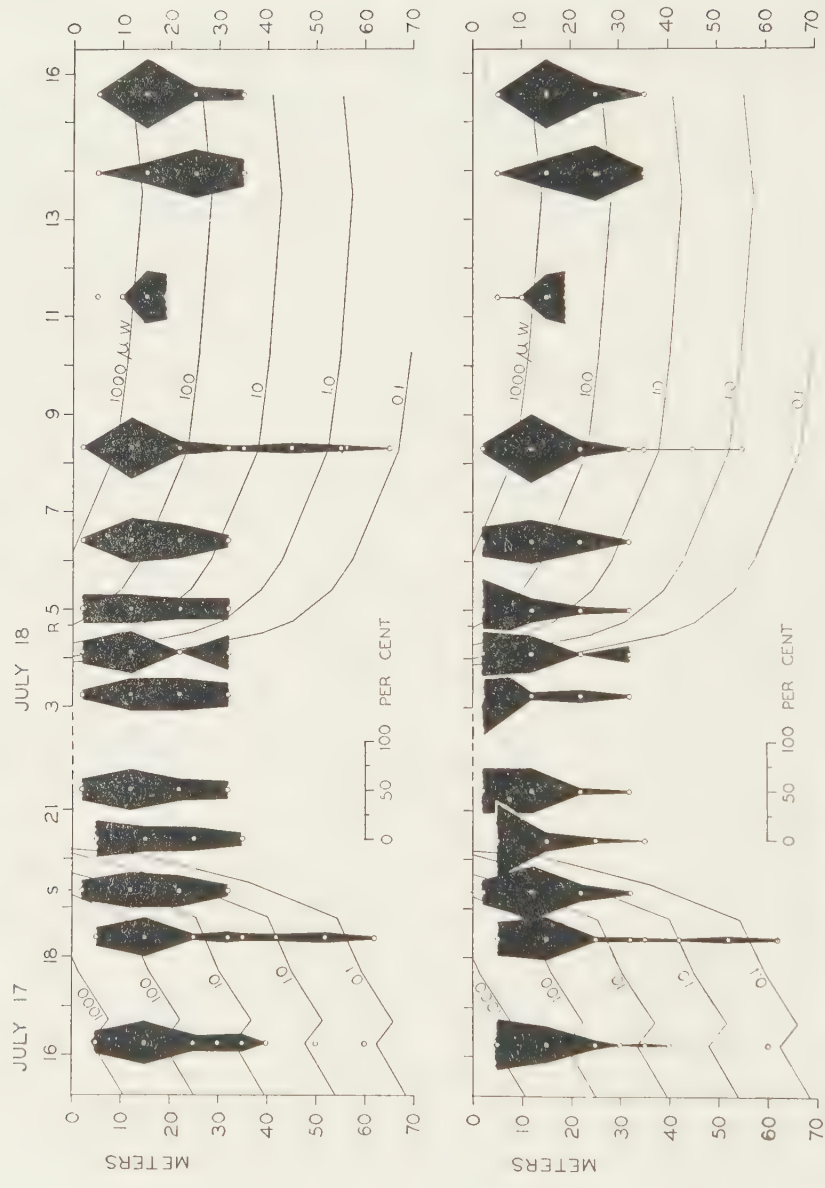

FIG. 4. Upper—*Calanus finmarchicus*, adult male. Lower—*Calanus finmarchicus*, adult female. The diurnal migration during the 24-hour period at the Bank station (Station 1723).

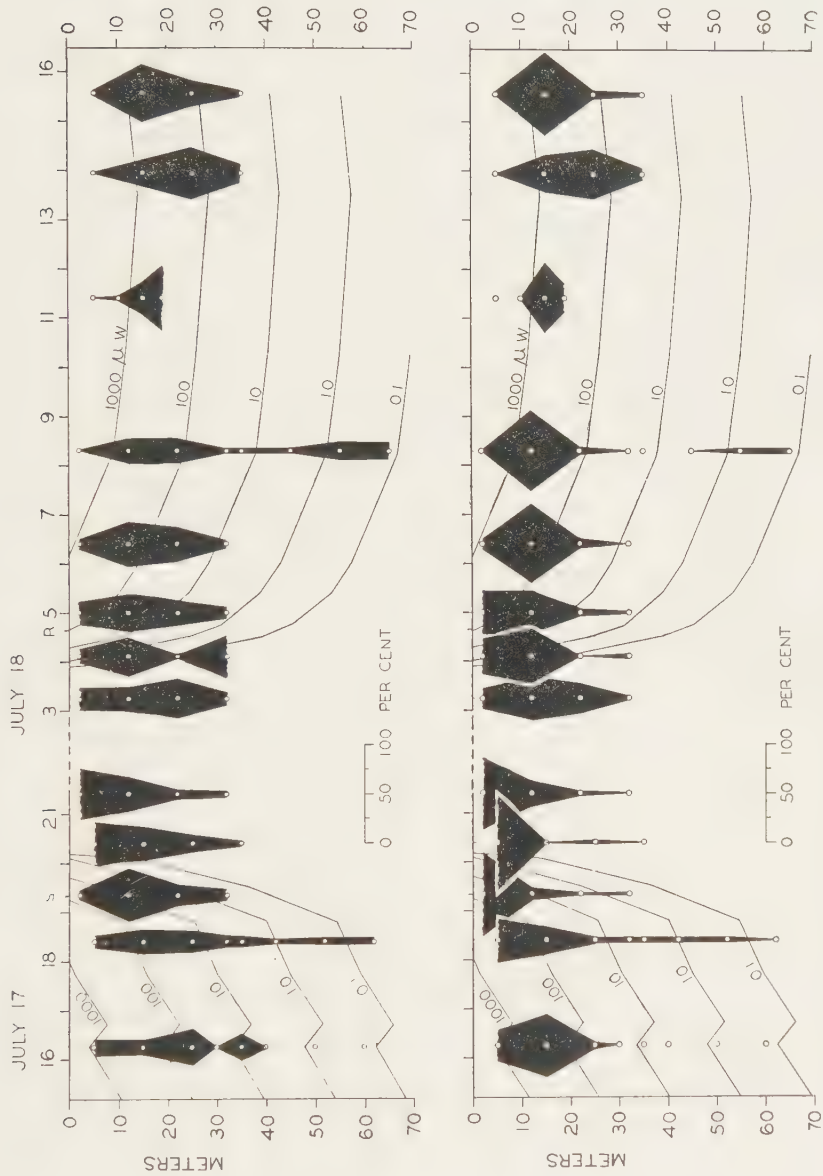

FIG. 5. Upper—*Calanus finmarchicus*, copepodid stage V. Lower—*Calanus finmarchicus*, copepodid stage IV. The diurnal migration at the Bank station (Station 1723).

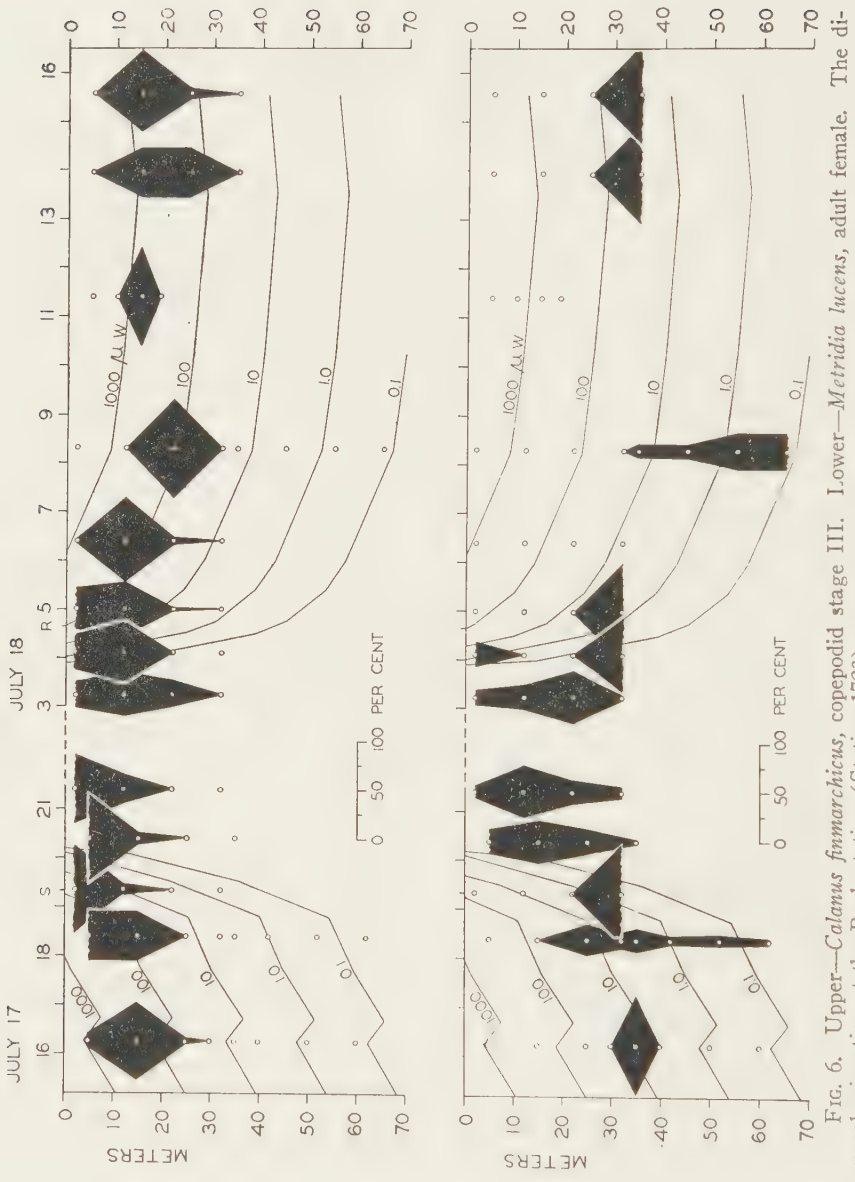

FIG. 6. Upper—*Calanus finmarchicus*, copepodid stage III. Lower—*Metridia lucens*, adult female. The diurnal migration at the Bank station (Station 1723).

surface at night than stage V and did not descend as far during the day. The tendency toward uniform distribution early in the morning is clearly marked. The distribution of copepodid stage III (Fig. 6, upper) was much the same as stage IV except that the level of maximum abundance never occurred below 22 meters and extremely few individuals were taken below 25 meters at any time.

While the *Calanus* groups were carrying out these very limited migrations at this station, the *Metridia* population showed a considerable vertical movement. During the first afternoon and evening the level of maximum abundance of the adult females (Fig. 6, lower) moved upwards from 35 meters to 12 meters. The animals did not continue migrating toward the surface during the night as previously (Clarke, 1933), for early the next morning the maximum occurred at 22 meters. A rapid downward movement followed until at 0830 the population was found chiefly below 35 meters. The adult males and immature of *Metridia,* which were taken in small numbers, appear to have migrated in much the same manner as the adult females, although not as extensively.

Since all the groups of *Calanus* remained in the upper water layers throughout the day at this station the animals must have been able to withstand strong irradiation. It can be seen from the figures that the copepods sometimes occurred in large numbers at levels where the light intensity was 1000 μw or more. However, the maximum abundance for all the groups of *Calanus* was usually found between the 1000 μw and the 100 μw lines. In most cases it is evident that the animals are inhabiting zones of higher light intensity in the afternoon than early in the morning, indicating that light-adaptation has taken place. The adult females of *Metridia* were confined to zones of lower irradiation. In the morning they appear to have avoided light intensities higher than 10 μw, but later in the day they were taken at levels where the irradiation was between 100 μw and 10 μw.

Discussion

The present observations illustrate the fact frequently pointed out by other investigators that the diurnal migration of plankton is a highly variable affair (Russell, 1932; Southern and Gardiner, 1932). The fact that the sex and age groups of *Calanus finmarchicus* all exhibited much the same type of behavior in the present case is in sharp contrast with the observations of Nicholls (1933) on this species made during his excellent investigations in the Clyde Sea-Area. At our deep station *Calanus* was found to be carrying out an extensive migration but at our

Bank station the same species exhibited a very slight diurnal movement. At the deep station the temperature was generally lower than at the Bank station, there being about 2° C. difference at the surface. Since *Calanus* is a cold-water species one would have expected a movement away from the surface in the warmer locality rather than in the colder. At the deep station the copepods carried out their migration in spite of the vertical oscillations of the water strata. The fact that the animals moved across the thermocline shows that in this case, at least, temperature is of minor importance in controlling vertical distribution (*cf.* Clarke, 1934*b*; Russell, 1927, p. 241). At the Bank station the copepods remained in large measure above the thermocline.

The salinity of the upper strata was slightly lower at the deep station than at the Bank station, but below 30 meters it was much the same at both localities. There was very little difference in other hydrographic conditions at the two stations (Braarud, 1934) and the differences in the phytoplankton do not appear to be significant although we do not know precisely which forms are important as food organisms (*cf.* Clarke, 1934*a*). The dominant form, *Pontosphæra,* was abundant above the thermocline in both cases, and the diatom population below the thermocline at the Bank station can be of no importance since the majority of the animals did not visit the lower strata. In view of this situation not only does it appear that the differences in the behavior at the two stations are not due to differences in the phytoplankton, but also it is difficult to understand how food could be an important factor in causing or controlling the diurnal migration which did occur at the deep station (*cf.* Worthington, 1931; Nicholls, 1933). In their upward movement the animals passed right through the zone of abundance of phytoplankton.

The year before in the Gulf of Maine at Stations 1285 and 1286 (Clarke, 1933) the adult females of *Calanus* exhibited no regular movement and copepodid stages V and IV migrated over a very limited range only. At Station 1287 the adult female population tended to rise and fall between 16 and 30 meters, but the copepodid stages showed very little tendency to migrate. In general in both cases the females and stages V and IV occurred in nearly equal numbers at all depths. The environmental conditions at the time when these observations were made were not strikingly dissimilar from those at Stations 1722 and 1723.

Metridia carried out distinct migrations at both Stations 1722 and 1723, although in the former case the vertical movement was more extensive. The species—particularly the adult females—is evidently more sensitive to light than *Calanus* since it was confined to zones of less intense irradiation. Furthermore *Metridia* arrived at the surface

later than *Calanus* and left the surface earlier at both stations. The migration of the adult females of *Metridia* was generally similar to that observed for this group the year before at Station 1287 but differed in certain details. At Stations 1722 and 1723 the copepods did not show a tendency to continue swimming toward the surface after complete darkness as they did on the previous occasion. Moreover, in the present case the downward migration was begun more than an hour before sunrise, and the animals descended during the day to much greater depths at Station 1722 than they did at Station 1287 where the water was correspondingly deep.

These differences in behavior must represent differences in the physiological condition of the organisms. In the case of the wide disparity in the distribution and migration of *Calanus* at the deep station and the Bank station, the conclusion seems inevitable that a fundamental difference in the reaction of the animals to light existed. It may be that we are dealing with two distinct broods, which differed in their physiological states (*cf.* Russell, 1928, 1934), although no clear indication of this appears from the length measurements. On the other hand, dissimilarities in the response to light might have been caused within comparable broods by the differences in the environmental conditions even though these are apparently slight. It is significant to note, however, that nothing in the environment at the Bank station prevented *Metridia* from carrying out a considerable migration. Although it is well known that differences in the environment often affect the reactions of different organisms in diverse ways, the present state of our knowledge of the specific action of external factors on copepods is insufficient to explain the variations in these observations on diurnal migration. However, we do know that phototropic reactions are sometimes under a very delicate control (Clarke, 1932).

We may conclude that in the control of the diurnal migration of copepods the environmental conditions are important from two points of view. First, they have a direct action in providing the stimuli by which the animals' swimming reactions may be evoked. Secondly, an indirect action is involved by which the factors of the environment influence the physiological condition of the organism. The environment is responsible for the physiological differences which may exist not only between seasonal broods but also between individuals of the same brood. Of the factors which directly affect the vertical movement of the copepods, light is the most important, but the migration may be indirectly influenced by the environment if conditions are such that the animals' reactions to light and to the other factors are changed.

Summary

1. Observations were made on the diurnal migration of copepods in relation to submarine irradiation and other external factors at a 54-hour and a 24-hour station in the Gulf of Maine using the same methods as previously. The zoöplankton consisted almost entirely of *Calanus finmarchicus* with a small number of *Metridia lucens*. The length of the copepods varied much less than was the case previously.

2. The variation with depth of the light intensity, the temperature, chemical constituents, and the phytoplankton was observed. During the first station the temperature was found to fluctuate widely.

3. At the station in the deep part of the Gulf all groups of *Calanus* and *Metridia* migrated to a depth of 120 meters or more during the daytime and moved upwards to levels of from 6 meters to 42 meters at night. At the station on Georges Bank *Calanus* was confined to the surface strata, undergoing only a very limited migration, but *Metridia* carried out an extensive migration.

4. The migration of the copepods was found to be more closely correlated with the changes in the submarine irradiation than with changes in the hydrographic conditions or in the phytoplankton. However, great variability in the behavior was observed. This appears to be due to differences in the physiological condition of the animals and represents the indirect action of the environment.

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TABLE I

Station 1722, Lat. 42° 50′ N., Long. 67° 05′ W.

Hauls *A*301–*A*320: July 14, 1933; sea: slight; sky: overcast; sunset: 1927.

Hauls *A*322–*A*360: July 15, 1933; sea: slight; sky: few clouds, fog; sunrise: 0433; sunset: 1926.

Hauls *A*361–*A*403: July 16, 1933; sea: slight becoming smooth; sky: overcast; sunrise: 0434; sunset: 1926.

Temperature: (See also Fig. 1) 54 meters, 6.50° C.; 72 meters, 6.25° C.; 90 meters, 5.57° C.; 113 meters, 5.74° C.; 136 meters, 6.01° C.; 158 meters, 6.53° C.; 181 meters, 6.90° C.; 202 meters, 7.06° C.; 227 meters, 7.02° C.

Numbers of copepods in hundreds; x = less than 50.

The data for each haul are given in the following order: haul number, depth (meters), local sun time; irradiation at surface (μw/cm.^{2}), irradiation at haul depth (μw/cm.^{2}), irradiation 30 minutes before (%): *Calanus finmarchicus* adult male, adult female, cop. V, cop. IV, cop. III; *Metridia lucens* adult male, adult female, immature.

*A*301, 6, 1706; 4000, 1900, 140: x, x, x, x, 0; 0, 0, 0
*A*302, 18, 1706; 4000, 390, 140: x, x, x, x, 0; 0, 0, 0
*A*303, 30, 1706; 4000, 110, 140: x, x, x, x, 0; 0, 0, 0
*A*304, 42, 1706; 4000, 34, 140: x, x, x, x, 0; 0, 0, 0
*A*305, 54, 1706; 4000, 8.4, 140: x, x, x, x, 0; 0, 0, 0
*A*306, 66, 1759; 1700, 1.0, 170: x, x, 3, 2, 0; 0, 0, 0
*A*307, 78, 1759; 1700, —, 170: 1, 5, 17, 3, 0; 0, 0, 0
*A*308, 90, 1759; 1700, —, 170: 2, 15, 46, 5, 0; 0, 0, 0
*A*309, 102, 1759; 1700, —, 170: 3, 22, 90, 6, 0; 0, 0, 0
*A*310, 114, 1759; 1700, —, 170: 6, 39, 141, 6, 0; 0, 0, 0
*A*311, 66, 1936; 44, —, 770: 3, 2, 3, 2, 0; 3, 15, 6
*A*312, 78, 1936; 44, —, 770: 3, 1, 2, 2, x; 3, 11, 10
*A*314, 102, 1936; 44, —, 770: 5, 1, 4, 2, 0; 7, 29, 3
*A*315, 114, 1936; 44, —, 770: 3, 3, 12, 3, x; 2, 10, 19
*A*316, 66, 2139; 0, 0, no light: x, x, x, x, 0; x, x, x
*A*317, 78, 2139; 0, 0, no light: 1, x, 6, 1, 0; 1, 1, 2
*A*318, 90, 2139; 0, 0, no light; 1, 1, 3, 1, 0; 1, 5, 2
*A*319, 102, 2139; 0, 0, no light: x, 1, 3, 1, 0; x, x, 1
*A*320, 114, 2139; 0, 0, no light: 5, 14, 50, 3, 0; 3, 8, 9
*A*322, 18, 0429; 140, 14, 6: x, x, x, 0, 0; 0, x, 0
*A*323, 30, 0429; 140, 3.8, 6: 1, 1, 7, 1, 0; 0, 0, 0
*A*324, 42, 0429; 140, 0.9, 6: 7, 66, 697, 24, 0; 0, 0, 0
*A*325, 54, 0429; 140, 0.3, 6: 3, 79, 366, 17, 0; 0, 0, 0
*A*326, 18, 0531; 1300, 130, 46: x, x, 1, x, 0; 0, 0, 0
*A*327, 30, 0531; 1300, 35, 46: x, x, 1, x, 0; 0, 0, 0
*A*328, 42, 0531; 1300, 11, 46: 0, 0, 0, 0, 0; 0, 0, 0
*A*329, 54, 0531; 1300, 2.7, 46: 1, 2, 24, 1, 0; 0, 0, 0
*A*330, 66, 0531; 1300, 0.7, 46: 2, 10, 137, 6, 0; 0, 0, 0
*A*331, 66, 0859; 7100, 4.0, 86: 0, 0, x, x, 0; 0, 0, 0
*A*332, 78, 0859; 7100, 1.1, 86: x, x, x, x, 0; 0, 0, 0
*A*333, 90, 0859; 7100, —, 86: 0, x, x, x, 0; 0, 0, 0
*A*334, 102, 0859; 7100, —, 86: 0, x, x, x, 0; 0, 0, 0
*A*335, 114, 0859; 7100, —, 86: x, x, 3, 1, 0; 0, 0, 0
*A*336, 66, 1558; 6200, 3.5, 130: 0, x, x, x, 0; 0, 0, 0
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*A*339, 102, 1558; 6200, —, 130: 0, 53, 263, 3, 0; 0, 0, 0
*A*340, 114, 1558; 6200, —, 130: 3, 121, 283, 7, 0; 0, 0, 0
*A*341, 126, 1652; 4700, —, 140: 3, 24, 56, 6, 0; 0, 0, 0
*A*342, 138, 1652; 4700, —, 140: 2, 10, 44, 3, 0; 1, 7, 10

TABLE I—*Continued*

*A*343, 150, 1652; 4700, —, 140 : 3, 22, 47, 3, 0; 2, 7, 5
*A*344, 162, 1652; 4700, —, 140 : 7, 10, 41, 6, 0; 4, 7, 4
*A*345, 174, 1652; 4700, —, 140 : 3, 14, 93, 7, 0; 5, 3, 10
*A*346, 66, 1858; 810, 0.5, 150 : x, 117, 1079, 23, 0; 0, 0, 0
*A*347, 78, 1858; 810, —, 150 : 4, 57, 486, 23, 0; 0, 0, 4
*A*348, 90, 1858; 810, —, 150 : 3, 41, 251, 14, 0; 0, 0, 0
*A*349, 102, 1858; 810, —, 150 : 3, 17, 283, 14, 0; 0, 0, 3
*A*350, 114, 1858; 810, —, 150 : 7, 83, 258, 10, 0; 3, 3, 17
*A*351, 6, 2009; 3.9, 1.8, 610 : 2, 23, 76, 2, 0; 0, 0, 0
*A*353, 30, 2009; 3.9, 0.1, 610 : x, 375, 2580, 42, 0; 0, 0, 0
*A*354, 42, 2009; 3.9, —, 610 : 10, 104, 262, 41, 0; 0, 35, 10
*A*355, 54, 2009; 3.9, —, 610 : 7, 104, 217, 10, 0; 3, 210, 35
*A*356, 6, 2138; 0, 0, no light : 4, 164, 312, 11, 0; 0, 8, 4
*A*357, 18, 2138; 0, 0, no light : 0, 61, 410, 27, 0; 0, 4, 0
*A*358, 30, 2138; 0, 0, no light : x, 150, 1855, 60, 0; 0, 0, 0
*A*359, 42, 2138; 0, 0, no light : 8, 163, 627, 15, 0; 4, 76, 53
*A*360, 54, 2138; 0, 0, no light : 8, 68, 213, 8, 0; 4, 125, 38
*A*361, 6, 0342; 0, 0, no light : x, 126, 14, 0, 0; 0, 2, 0
*A*362, 18, 0342; 0, 0, no light : 2, 111, 109, 0, 0; 0, 0, 0
*A*364, 42, 0342; 0, 0, no light : 11, 110, 567, 8, 0; 0, 4, 4
*A*365, 54, 0342; 0, 0, no light : x, 6, 32, x, 0; 0, 0, 0
*A*366, 6, 0434; 78, 39, 3 : 1, 12, 25, 0, 0; 0, 0, 0
*A*367, 18, 0434; 78, 7.8, 3 : x, 7, 3, x, 0; 0, 0, 0
*A*368, 30, 0434; 78, 1.2, 3 : x, 28, 25, x, 0; 0, 0, 0
*A*369, 42, 0434; 78, 0.3, 3 : x, 19, 5, 0, 0; 0, 0, 0
*A*370, 54, 0434; 78, —, 3 : x, 21, 9, x, 0; 0, 0, 0
*A*372, 66, 0539; 1300, 0.7, 26 : 0, x, x, x, 0; 0, 0, 0
*A*373, 78, 0539; 1300, —, 26 : x, 2, 6, x, 0; 0, 0, 0
*A*374, 90, 0539; 1300, —, 26 : x, 5, 17, 1, 0; 0, 0, 0
*A*375, 102, 0539; 1300, —, 26 : 1, 19, 39, 3, 0; 0, 0, 0
*A*376, 66, 0702; 2800, 1.6, 61 : 0, x, 1, x, 0; 0, 0, 0
*A*377, 90, 0702; 2800, 0.1, 61 : x, 1, 4, 1, 0; 0, 0, 0
*A*378, 114, 0702; 2800, —, 61 : 1, 17, 83, 11, 0; 0, 0, 0
*A*379, 138, 0702; 2800, —, 61 : 15, 72, 349, 11, 0; 0, 4, 15
*A*380, 66, 0902; 7500, 4.3, 87 : x, x, x, x, 0; 0, 0, 0
*A*381, 90, 0902; 7500, 0.3, 87 : x, x, x, x, 0; 0, 0, 0
*A*382, 114, 0902; 7500, —, 87 : 0, x, x, x, 0; x, 0, x
*A*383, 138, 0902; 7500, —, 87 : 4, 4, 95, 23, 0; 6, 2, 21
*A*384, 6, 1547; 3900, 2000, 110 : x, x, x, 0; 0, 0, 0
*A*385, 18, 1547; 3900, 390, 110 : x, 2, 1, x, 0; 0, 0, 0
*A*386, 30, 1547; 3900, 59, 110 : x, x, x, x, 0; 0, 0, 0
*A*387, 42, 1547; 3900, 15, 110 : x, x, x, x, x; 0, 0, 0
*A*388, 66, 1628; 2800, 1.6, 130; 0, 48, 149, 4, 0; 0, 0, 0
*A*389, 90, 1628; 2800, 0.1, 130 : 8, 144, 353, 4, 0; 0, 4, 8
*A*390, 114, 1628; 2800, —, 130 : 4, 171, 281, 4, 0; 4, 4, 4
*A*391, 138, 1628; 2800, —, 130 : 2, 14, 73, 3, 0; 2, 7, 12
*A*392, 18, 1743; 1200, 120, 200 : x, 2, 5, 1, x; 0, 1, x
*A*393, 42, 1743; 1200, 4.6, 200 : 3, 14, 47, 4, 0; 0, x, 1
*A*394, 66, 1743; 1200, 0.7, 200 : 15, 80, 433, 11, 0; 0, 0, 0
*A*395, 90, 1743; 1200, —, 200 : 36, 320, 515, 18, 0; 0, 0, 0
*A*396, 18, 1844; 910, 91, 130 : 3, 57, 85, 2, 0; 0, 0, 0
*A*397, 42, 1844; 910, 3.5, 130 : 7, 148, 248, 7, 0; 0, 0, 0
*A*398, 66, 1844; 910, 0.5, 130 : 19, 281, 247, 4, 0; 0, 4, 11
*A*399, 92, 1844; 910, —, 130 : 10, 30, 36, 6, 0; 3, 23, 17
*A*400, 18, 1948; 18, 1.8, 1300 : 7, 221, 186, 7, 0; 0, 0, 0
*A*401, 30, 1948; 18, 0.3, 1300 : 15, 426, 643, 19, 4; 0, 0, 0
*A*402, 42, 1948; 18, —, 1300 : 24, 345, 322, x, 0; 0, x, 12
*A*403, 54, 1948; 18, —, 1300 : 15, 274, 293, 11, 0; 0, 42, 19

Table II

Station 1723, Lat. 41° 20′ N., Long. 66° 40′ W.

Hauls *A*404–*A*431, July 17, 1933, Sea: smooth, heavy swell; sky: clear, fog; sunset: 1920.

Hauls *A*432–*A*467, July 18, 1933; sea: smooth, becoming slight, moderate swell; sky: few clouds, fog; sunrise: 0440; sunset: 1919.

Temperature: 1 meter, 15.45° C.; 5 meters, 15.47° C.; 10 meters, 15.39° C.; 15 meters, 13.92° C.; 20 meters, 10.34° C.; 25 meters, 8.67° C.; 30 meters, 8.47° C.; 40 meters, 8.44° C.; 50 meters, 8.21° C.; 60 meters, 8.07° C.; 70 meters, 7.98° C.

Numbers of copepods in hundreds; x = less than 50.

The data for each haul are given in the following order: Haul number, depth (meters), local suntime; irradiation at surface (μw/cm.^{2}), irradiation at haul depth (μw/cm.^{2}), irradiation 30 minutes before (%): *Calanus finmarchicus,* adult male, adult female, cop. V, cop. IV, cop. III; *Metridia lucens,* adult male, adult female, immature.

*A*404, 5, 1558; 3200, 1400, —: 10, 76, 31, 10, 2; 0, 0, 0
*A*405, 15, 1558; 3200, 190, —: 20, 55, 30, 20, 11; 0, 0, 0
*A*406, 25, 1558; 3200, 35, —: 8, 13, 65, 2, 1; 0, 0, 0
*A*407, 35, 1558; 3200, 7.0, —: 8, 4, 47, 0, 0; 4, 35, 5
*A*408, 30, 1632; 3700, 22, 87: x, x, x, x, 0; 0, x, 0
*A*409, 40, 1632; 3700, 4.8, 87: x, x, x, x, x; x, x, x
*A*410, 50, 1632; 3700, 1.0, 87: x, x, x, x, x; 0, 0, 0
*A*411, 60, 1632; 3700, 0.2, 87: x, x, x, x, 0; 0, 0, 0
*A*412, 5, 1759; 1400, 600, 120: 11, 34, 32, 8, 3; 0, 0, 0
*A*413, 15, 1759; 1400, 83, 120: 23, 42, 69, 5, 3; 0, 0, 0
*A*414, 25, 1759; 1400, 15, 120: 3, 4, 58, 1, 0; 0, 14, 1
*A*415, 35, 1759; 1400, 3.1, 120: 4, 2, 31, 1, 0; 0, 10, x
*A*416, 32, 1847; 650, 2.3, 170: 6, 4, 33, 1, 0; 0, 8, 0
*A*417, 42, 1847; 650, 0.7, 170: 4, 2, 14, 1, x; 8, 4, 1
*A*418, 52, 1847; 650, 0.1, 170: 6, 6, 19, 1, 0; 10, 5, x
*A*419, 62, 1847; 650, —, 170: 1, 2, 20, x, 0; 3, 3, 1
*A*420, 2, 1921; 99, 56, 530: 14, 86, 24, 77, 24; 0, 0, 0
*A*421, 12, 1921; 99, 13, 530: 25, 192, 143, 12, 3; 0, 0, 0
*A*422, 22, 1921; 99, 1.7, 530: 22, 61, 58, 1, 0; 0, 0, 0
*A*423, 32, 1921; 99, 0.3, 530: 8, 10, 33, 1, 0; 0, 12, 0
*A*424, 5, 2024; 0, 0, (5.2 μw): 14, 183, 121, 66, 14; 0, 7, 0
*A*425, 15, 2024; 0, 0, (5.2 μw): 10, 47, 99, 0, 2; 0, 12, 0
*A*426, 25, 2024; 0, 0, (5.2 μw): 9, 13, 47, 2, 0; 0, 7, 1
*A*427, 35, 2024; 0, 0, (5.2 μw): 5, 2, 15, x, 0; x, 1, 1
*A*428, 2, 2126; 0, 0, no light: 7, 87, 125, 34, 12; 2, 2, 0
*A*429, 12, 2126; 0, 0, no light: 14, 88, 85, 12, 5; 0, 9, 0
*A*430, 22, 2126; 0, 0, no light: 7, 10, 21, 1, x; 1, 4, 0
*A*431, 32, 2126; 0, 0, no light: 6, 3, 15, x, 0; x, 2, 0
*A*432, 2, 0315; 0, 0, no light: 3, 245, 52, 7, 3; 0, 3, 0
*A*433, 12, 0315; 0, 0, no light: 11, 23, 55, 15, 5; 0, 5, 0
*A*434, 22, 0315; 0, 0, no light: 11, 36, 91, 10, 3; 0, 10, 0
*A*435, 32, 0315; 0, 0, no light: 8, 6, 29, 1, 0; 1, 1, 0
*A*436, 2, 0406; 2.6, 1.5, no light: 3, 32, 7, 7, 5; 0, 1, 0
*A*437, 12, 0406; 2.6, 0.3, no light: 6, 27, 19, 11, 8; 0, 0, 0
*A*438, 22, 0406; 2.6, —, no light: x, 1, 1, x, 0; 0, x, 0
*A*439, 32, 0406; 2.6, —, no light: 5, 12, 20, 1, 0; 1, 3, 1
*A*440, 2, 0501; 380, 210, 16: 4, 75, 13, 31, 20; 0, 0, 0

TABLE II—*Continued*

*A*441, 12, 0501; 380, 49, 16: 4, 30, 22, 31, 26; 0, 0, 0
*A*442, 22, 0501; 380, 6.5, 16: 3, 10, 15, 5, 1; 0, 0, 0
*A*443, 32, 0501; 380, 1.3, 16: 3, 4, 7, 1, x; x, 1, x
*A*444, 2, 0626; 1600, 900, 81: 1, 26, 8, 3, 1; 0, 0, 0
*A*445, 12, 0626; 1600, 210, 81: 8, 40, 36, 25, 13; 0, 0, 0
*A*446, 22, 0626; 1600, 28, 81: 6, 20, 28, 3, 1; 0, 0, 0
*A*447, 32, 0626; 1600, 5.6, 81: 2, 2, 6, x, x; 0, 0, 0
*A*448, 2, 0802; 4900, 2800, 76: 0, 7, x, x, 0; 0, 0, 0
*A*449, 12, 0802; 4900, 640, 76: 13, 53, 30, 18, 6; 0, 0, 0
*A*450, 22, 0802; 4900, 85, 76: 3, 13, 31, 2, 0; 0, 0, 0
*A*451, 32, 0802; 4900, 17, 76: 1, 1, 5, 0, 0; 0, 0, 0
*A*452, 35, 0836; 6200, 14, 79: 1, x, 6, x, x; 1, 1, x
*A*453, 45, 0836; 6200, 4.0, 79: 2, 1, 5, x, 0; 1, 1, 1
*A*454, 55, 0836; 6200, 0.7, 79: 1, 1, 23, 1, x; 1, 3, 2
*A*455, 65, 0836; 6200, 0.1, 79: x, x, 22, 1, 0; 2, 3, 1
*A*456, 5, 1126; 11,500, 4900, 88: 0, x, x, x, 0; 0, 0, 0
*A*457, 10, 1126; 11,500, 2300, 88: 0, 1, 1, x, 0; 0, 0, 0
*A*458, 15, 1126; 11,500, 680, 88: 25, 60, 130, 15, 12; 0, 0, 0
*A*459, 19, 1126; 11,500, 320, 88: 22, 69, 259, 6, 0; 0, 0, 0
*A*460, 5, 1357; 10,800, 4600, 120: x, x, 0, 0, 0; 0, 0, 0
*A*461, 15, 1357; 10,800, 640, 120: 6, 30, 46, 6, 1; 0, 0, 0
*A*462, 25, 1357; 10,800, 120, 120: 14, 58, 84, 8, 1; 0, 0, 0
*A*463, 35, 1357; 10,800, 24, 120: 8, 11, 30, 2, 0; 0, 1, 0
*A*464, 5, 1535; 9000, 3800, 110: 1, 7, 12, 1, x; 0, 0, 0
*A*465, 15, 1535; 9000, 530, 110: 28, 91, 97, 28, 8; 0, 0, 0
*A*466, 25, 1535; 9000, 100, 110: 6, 28, 44, 3, 1; 0, 0, 0
*A*467, 35, 1535; 9000, 20, 110: 4, 3, 11, 1, x; 0, 1, x

Table III

Cephalothorax lengths of Calanus finmarchicus

	Station 1722									Station 1723							
	Day Hauls						Night Hauls			Day Hauls					Night Hauls		
	Haul 384 6 m.	Haul 386 30 m.	Haul 336 66 m.	Haul 339 102 m.	Haul 342 138 m.	Haul 345 174 m.	Haul 356 6 m.	Haul 358 30 m.	Haul 360 54 m.	Haul 404 5 m.	Haul 405 15 m.	Haul 408 30 m.	Haul 409 40 m.	Haul 411 60 m.	Haul 428 2 m.	Haul 429 12 m.	Haul 431 32 m.
Adult male																	
No measured	—	—	—	—	—	—	—	—	—	25	25	—	9	6	16	24	25
Average length mm.	—	—	—	—	—	—	—	—	—	2.506	2.504	—	2.508	2.416	2.553	2.455	2.514
Range	—	—	—	—	—	—	—	—	—	2.295–2.635	2.380–2.720	—	2.423–2.678	2.338–2.508	2.380–2.720	2.253–2.635	2.210–2.720
Adult female																	
No. measured	—	—	—	24	25	25	25	25	25	25	25	3	17	26	53	25	26
Average length mm.	—	—	—	2.722	2.710	2.693	2.654	2.715	2.669	2.581	2.606	2.621	2.588	2.536	2.674	2.640	2.621
Range	—	—	—	2.465–3.103	2.465–2.975	2.380–3.103	2.465–2.975	2.380–3.018	2.380–3.060	2.210–2.848	2.423–2.805	2.465–2.720	2.380–2.805	2.380–2.720	2.380–3.018	2.465–3.145	2.423–2.933

TABLE III—*Continued*

	Station 1722									Station 1723							
	Day Hauls						Night Hauls			Day Hauls					Night Hauls		
	Haul 384 6 m.	Haul 386 30 m.	Haul 336 66 m.	Haul 339 102 m.	Haul 342 138 m.	Haul 345 174 m.	Haul 356 6 m.	Haul 358 30 m.	Haul 360 54 m.	Haul 404 5 m.	Haul 405 15 m.	Haul 408 30 m.	Haul 409 40 m.	Haul 411 60 m.	Haul 428 2 m.	Haul 429 12 m.	Haul 431 32 m.
Copepodid V																	
No. measured	5	26	25	50	52	50	50	49	50	50	50	21	11	12	100	51	50
Average length mm.	2.431	2.240	2.220	2.424	2.460	2.399	2.422	2.388	2.436	2.417	2.458	2.374	2.241	2.271	2.434	2.329	2.350
Range	2.125–2.720	2.040–2.465	2.040–2.423	2.168–2.720	2.125–2.720	2.125–2.763	2.125–2.763	2.083–2.593	2.125–2.720	2.168–2.720	2.168–2.635	1.998–2.593	1.955–2.423	1.998–2.465	1.998–2.720	2.040–2.720	1.955–2.635
Copepodid IV																	
No. measured	—	—	—	—	25	25	25	12	12	25	25	1	1	1	50	26	2
Average length mm.	—	—	—	—	1.903	1.874	1.875	1.885	1.853	1.826	1.867	1.785	1.743	1.785	1.845	1.849	1.807
Range	—	—	—	—	1.785–2.040	1.700–2.338	1.658–2.040	1.828–2.083	1.785–1.955	1.615–1.955	1.743–2.083	—	—	—	1.700–1.998	1.700–2.083	1.785–1.828

TABLE IV

Comparison of Cephalothorax length and total length in Calanus finmarchicus

	Haul No.	No. measured	Average cephalothorax length	Average total length	Ratio
			mm.	*mm.*	
Adult male	431	25	2.508	3.230	0.777
Adult female	431	25	2.635	3.315	0.795
" "	358	25	2.720	3.400	0.800
Copepodid V	358	50	2.338	2.933	0.797
" IV	358	13	1.743	2.168	0.805

Contribution No. 49

(Reprinted from Transactions of the American Geophysical Union,
Fifteenth Annual Meeting, 1934)

SURFACE-TEMPERATURES ON THE NEW YORK TO CAPE SÃO ROQUE STEAMSHIP-ROUTE

Phil E. Church[1)]

Early in 1932 the Brazilian Traction and Light Company installed thermographs on three of their vessels which ply between New York and Cape São Roque and thence to

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[1)] Contribution No. 49 from the Woods Hole Oceanographic Institution.

South American ports. Twenty-one southbound trips furnished the data for the preliminary survey of the line over which these vessels traveled. The period of investigation was from January 1932 through August 1933. The vessels plied over a great-circle route which, passing north of Bermuda, transversely cut across the continental shelf, the slope-area, and the Gulf Stream, and passed obliquely across the Central Atlantic area and the North Equatorial Current.

The accuracy of the instruments was of a high order and only infrequent adjustments were necessary, which were made by officials when the vessels were in either New York or Santos.

Only the Central Atlantic and North Equatorial Current areas will be discussed here because the surface-temperatures of the continental shelf, the slope-area, and the Gulf Stream have been treated in papers by Bigelow (H. B. Bigelow, Studies of the waters on the continental shelf, Cape Cod to Chesapeake Bay, Part I, The cycle of temperature, Papers in Physical Oceanography and Meteorology, published by Massachusetts Institute of Technology and Woods Hole Oceanographic Institution, 1933), Harwood and Brooks (E. M. Harwood and C. F. Brooks, Monthly sequence of sea-surface temperatures on the New York-San Juan steamship-route, Trans. Amer. Geophys. Union, 14th annual meeting, 1933, pp. 173-180), and Church (P. E. Church, Surface-temperatures of the Gulf Stream and its bordering waters, Geog. Rev., v. 22, No. 2, pp. 286-293; also Progress in the investigation of surface-temperatures of the western North Atlantic, Trans. Amer. Geophys. Union, 13th annual meeting, 1932, pp. 244-249).

Central Atlantic Water--South and east of the Gulf Stream lies that great, wide expanse of uniformly warm water. To the north and west its limit is sharply marked by the Gulf Stream, but its southern edge is most indefinite. Criteria other than surface-temperature will probably have to be employed in finally defining the exact limit, if an exact limit is to be made. Harwood and Brooks, on the New York to San Juan steamship-route, placed the southern edge at that point where there was no further increase in temperature with a decrease of latitude. They found that the Central Atlantic Water, on its western edge, was broader in winter than in summer. The lesser width in summer is ascribable, for the most part, to the more northerly position of the summer Sun. As the Central Atlantic loses in width, the Antilles Water to the south gains in width, and the loss in width of one is compensated for by a corresponding gain in width of the other. Only to a certain extent does this apply to the waters farther east where the Brazilian Traction and Light Company vessels cross. It is true that here the Central Atlantic Water is narrower in summer and wider in winter, but the corresponding loss or gain is not entirely made up from the next band of water to the south but from the next two bands. Also, the width of the Central Atlantic Water has a far greater seasonal fluctuation here than to the westward. If we may strike an average for the yearly position of the southern limit, using the criterion of Harwood and Brooks, it would be found at approximately latitude 24° north and longitude 53° west (longitude of Cape Race, Newfoundland). From this average position there is a shift of at least three degrees of latitude north or south, depending upon the season. The northern boundary lies somewhere near latitude 27° north in late summer (early September) and six months later this same boundary is found in the neighborhood of latitude 21° north. This shift of boundary of 300 to 400 miles in latitude cannot be laid to any great floodings of warmer surface-water; for the present it must be assumed to be the result of the shift of the Sun.

Winter temperatures--Minimum temperatures occur in late February or early March for this area as a whole. At this time the lowest temperatures are to be found just south of the Gulf Stream. Here the water is between 19° and 20° C. Southward there is a gradual increase in surface-warmth to approximately the parallel of 26° where a sudden rise of one degree or more brings the temperature to 23.5 or 24° C. From this position to the parallel of 21° there is an increase in temperature of only one degree and south of that the surface-warmth remains quite constant for at least another 300 miles more. The rise of temperature amounts to but five or six degrees within 16 degrees of latitude. We may consider the southern edge of this body at this season to be latitude 21° north.

Summer temperatures--Maximum temperatures are reached by the latter part of August or early September. Again the coolest water is just south of the Gulf Stream, north of Bermuda. Here the thermographs register 27° C, and fractionally higher

towards Cape Hatteras. To the south, at latitude 30° north the temperature is slightly above 28° C, and increases slowly to the highest temperatures along this route somewhere in the vicinity of latitude 27° north where the superficial layers may be from 29°.5 C to 30° C. The exact position of this high degree of heat varies somewhat from year to year, but a rise of two or three degrees is experienced in this body at this season in the latitudinal range of six to ten degrees.

Annual range--Nowhere over the western part of this body of water does the annual range exceed ten degrees. The annual range is greatest on the extreme western part (over the New York to San Juan route) where it is nearest the continent. North and east of Bermuda, on the New York to Cape São Roque route, the annual range is between eight and nine degrees, while south of the parallel of 30° it diminishes gradually and evenly to six degrees at latitude 24° north. It appears that this line of six degrees of annual range may tentatively be used as the southern boundary of the Central Atlantic Water. It coincides quite closely with the position of that boundary as formulated by Harwood and Brooks and also agrees largely with Schott's southern boundary of the Sargasso Sea where the weed Sargassum is "sehr hauf" (G. Schott, Geographie des Atlantischen Ozeans, Tafel 26, p. 264, 1912). However, this limit is considerably south of the limit of normal salinity (36.5) of the Central Atlantic Water which Iselin (C. O'D. Iselin, unpublished manuscript, 1933) states to be in the neighborhood of latitude 28° 30' north.

North Equatorial Current--South of the Central Atlantic Water on the New York to Cape São Roque route is the broad expanse known as the North Equatorial Current. It is at least 1500 miles wide, latitudinally, and is composed of a complex of at least three different types of water which can be distinguished on the basis of surface-temperature characteristics. These different bodies agree, in a large measure, with bands of different salinities. This leads to the assumption that the water of each comes from a somewhat different source, or has differing factors influencing the physical properties. From north to south the following belts are found: From the southern edge of the Central Atlantic Water to the vicinity of latitude 15° north; from latitude 15° north to approximately latitude 7° north where the annual regime of temperatures of the northern hemisphere undergoes a shift from the normal; south of latitude 7° north to Cape São Roque. These subdivisions will be identified as the "northern belt," the "central belt," and the "equatorial belt."

Northern belt--The outstanding surface-temperature characteristic of this subdivision is the almost total lack of latitudinal gradient at any one season. From north to south there is a rise of not more than one or one and a half degrees. This occurs in the winter for in summer the reverse is apt to be true, that is, the warmer water being on the northern edge of the belt. This northern edge has an annual range of about 6° during the year and about 4° on its southern edge (latitude 15° north). In late summer the maximum temperatures may reach 30° C next to the Central Atlantic Water and fractionally lower farther south. In late winter the northern edge cools to minimum of 25°.5 C and 26° C along its southern edge.

This northern belt corresponds to what Iselin (unpublished manuscript, 1933) has called a horse latitude, high salinity-belt. The high salinity (37) is thought to result from the great evaporation caused by high temperatures, little wind, and low humidity from the descending air of the North Atlantic (Azores-Bermuda) high-pressure belt.

Central belt--Latitudinally this belt is about 500 miles wide. Its northern edge is poorly defined as regards temperature, the only indication of a boundary occurring in the months of February, March, and April when there is a slight break in temperature of a half to a whole degree. A small change, such as this, would mean little or nothing in the water of the extra-tropics, but within the tropics where there is a small annual range, a distinct break in temperature is, perhaps, sufficient justification for use as a boundary. Furthermore, the water here corresponds to the trade-wind belt where the water is less salty (36.25) than that to the north. The southern edge of this belt is marked not only by a change in the annual march of temperature, but also a change in the salinity for the water is considerably fresher.

The annual range of temperature in this belt is even less than the northern belt for the range amounts to only 3° or 4° at most. This is owing, no doubt, to the lower

latitude of the central belt and also to the condition of water a degree or more warmer than the southern edge of the northern belt at the time the latter belt has reached its minimum temperature. The distinctly higher temperature of the central belt in late winter and the smaller annual range seem to indicate that the water of the central belt remains within the tropics for a longer period than that of the northern belt. The minimum occurs in February when the surface is between 25.°5 C and 26° C and the maximum comes in September when the temperature is 28.°5 C to 29° C. At the period of maximum temperature both the Central Atlantic Water and the northern belt may have a surface-temperature slightly higher than the central belt.

Equatorial belt--In the vicinity of the parallel 7° north of the equator northwest of Cape São Roque, there is a decided shift in the annual regime of surface-temperatures. Except from July through November, there is a sharp increase in surface-

warmth contrasted with the central belt. From July through November the temperature remains about the same as that to the north, but along the adjacent boundaries of the two subdivisions there are streaks of warmer and cooler water as suggested by the thermograph pen.

Annual march of temperature--As far south as latitude 2° north the minimum temperatures occur in early February and are followed by a slow warming until May or early June when a secondary maximum occurs. June, July, and August are somewhat cooler than the late spring maximum, but toward the end of summer warming is again evident. During October and November the temperature rises to another peak, this being the primary maximum. Near the northern edge of this belt the temperature may reach 29°5 C at this time. After the maximum has been attained, the drop in temperature is rapid until the minimum in February. The northern part of this belt has an annual range of from 2° to 2°5. Just north of the equator (about latitude 2° north) in the middle of this equatorial belt the annual range amounts to only 1°5 with a maximum of 28°5 in November.

Between the parallel 2° north of the equator and Cape São Roque the shift to the southern hemisphere temperature-regime is nearly completed. The maximum temperatures come in February, March, and April from which period the warmth slowly declines to a minimum during July, August, and September. Only a vestige of the November maximum exists. The annual range is slightly more here than in the belt to the north for it amounts to 2°5 to 3°. However, a lower minimum accounts for the greater annual range because the July-August temperatures get as low as 26° C or 26°5 C.

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Woods Hole, Massachusetts

Contribution No. 50

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THE EFFECT OF ACIDITY ON THE CARBON MONOXIDE-COMBINING POWER OF HEMOGLOBIN IN THE BLOOD OF MARINE FISHES*

By RAYMOND W. ROOT and ARDA ALDEN GREEN

(*From the Woods Hole Oceanographic Institution, Woods Hole*)

(Received for publication, June 21, 1934)

Previous studies on the effect of hydrogen ions on the equilibrium between hemoglobin and oxygen in the blood of a number of marine teleosts (Root, 1931; Green and Root, 1933) indicated rather strongly that acidity not only exerts the usual Bohr effect on these hemoglobins, but also causes them to lose a large fraction of their oxygen-combining power, even when they are exposed to an atmosphere of oxygen. It seemed probable, from the shape of the dissociation curves at acid reactions, that increasing the oxygen tension still further would not result in increasing the amount of oxygen combined with the hemoglobin—that is, that there is a true reversible inactivation of the respiratory pigment. Such inactivation might be due to the inability of the prosthetic group on the hemoglobin to combine with any gas in the presence of acid, or depend upon some particular behavior of the oxygen.

In human blood the dissociation curves for carboxyhemoglobin are essentially like those for oxyhemoglobin, except for the fact that carbon monoxide has a combining power some 245 times greater than that of oxygen (Douglas, Haldane, and Haldane, 1912). If such a situation exists with respect to fish hemoglobin, then data procured with the use of carbon monoxide ought to be comparable with data obtained by raising the oxygen tension by an amount proportional to the difference in combining power of the two gases, and, thus, show whether an increase in oxygen tension far above that used in previous work would increase the amount of oxygen bound in acid solutions. With this idea in mind a com-

* Contribution No. 50.

parison has been made of the behavior of fish hemoglobin in the presence of oxygen and carbon monoxide, especially at acid reactions.

Experiments were conducted with the bloods of the sea-robin, *Prionotus carolinus*, L., the tautog, *Tautoga onitis*, L., and the toadfish, *Opsanus tau*, L. The results show that there is no loss of carbon monoxide capacity in acid bloods. Moreover, the use of carbon monoxide is not exactly comparable with increasing the oxygen tension in proportion to the difference in their combining power, for the dissociation curves obtained with the two gases are not always similar in shape.

Methods

The technique of handling the blood was the same as described in the previous paper (Green and Root, 1933), except for the added procedure for the determination of carbon monoxide. All blood samples were equilibrated in a water bath at 25° and analyzed immediately in duplicate with the Van Slyke constant volume apparatus. The procedure described by Sendroy and Liu (1930) was used for the determination of carbon monoxide in the blood, the gas finally being absorbed by ammoniacal cuprous chloride solution. The gas in the tonometers was analyzed with the Haldane apparatus to which was attached a combustion chamber for the determination of carbon monoxide. The carbon monoxide was prepared from formic and sulfuric acids and stored for use over water.

All data expressing the amount of gas in the blood have been corrected for dissolved gas. An oxygen solubility coefficient of 2.70 volumes per cent per atmosphere (Green and Root, 1933) was used in calculating dissolved oxygen. Carbon monoxide solubility coefficients were experimentally determined for sea-robin and tautog bloods, the values obtained being 1.75 and 2.09 volumes per cent per atmosphere respectively. The value for tautog blood was assumed to hold for toadfish blood.

The pH of the blood has been calculated from gasometric data, the Henderson-Hasselbalch equation being used, assuming pK'_1 equal to 6.22 for whole blood at 25°. Bohr's (1905) solubility coefficient was adopted for the determination of dissolved carbon dioxide. In a number of cases the pH was measured with the glass

electrode. The values obtained by measurement averaged about 0.06 pH higher than the calculated values and indicate that some adjustment should be made in the constants of the equation. However, since constancy of pH is more important than the absolute values in this problem, no attempt has been made to evaluate new constants. The calculated pH value is given in each case, thus making it directly comparable with previous data on these bloods.

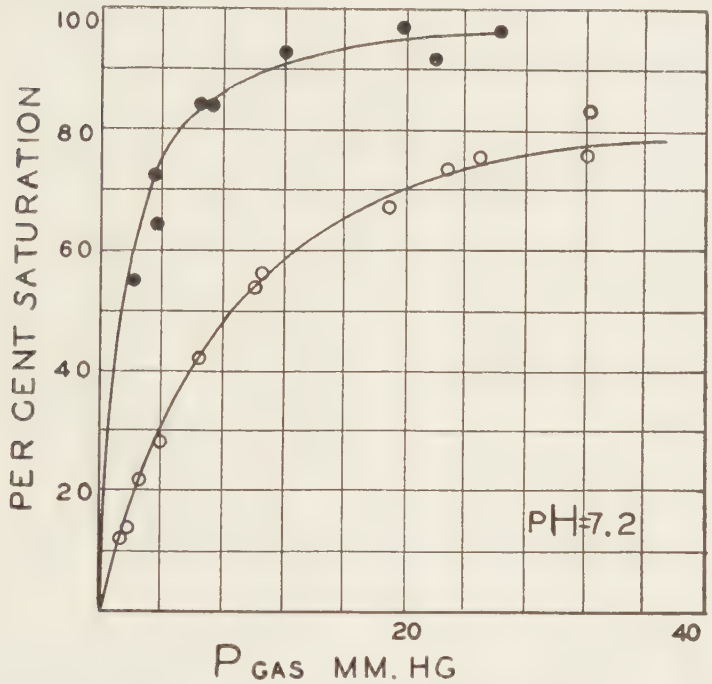

Fig. 1. The carbon monoxide (upper) and oxygen (lower) dissociation curves for sea-robin blood at pH 7.2. The oxygen pressure for each point on the oxygen curve has been divided by 25. The curves drawn through the points are rectangular hyperbolas. The dissociation constant for the carbon monoxide is actually 165 times greater than that for the oxygen curve.

Carbon Monoxide and Oxygen Dissociation Curves for Sea-Robin Blood

Because no data were available for the oxygen dissociation curves at 25°, both oxygen and carbon monoxide curves were

established at comparable pH values for this blood. The data are summarized in Figs. 1 to 4.

The curves established at pH 7.2 are similar, both capable of being described by rectangular hyperbolas with no reversible inactivation of the hemoglobin. The constant for the carbon mon-

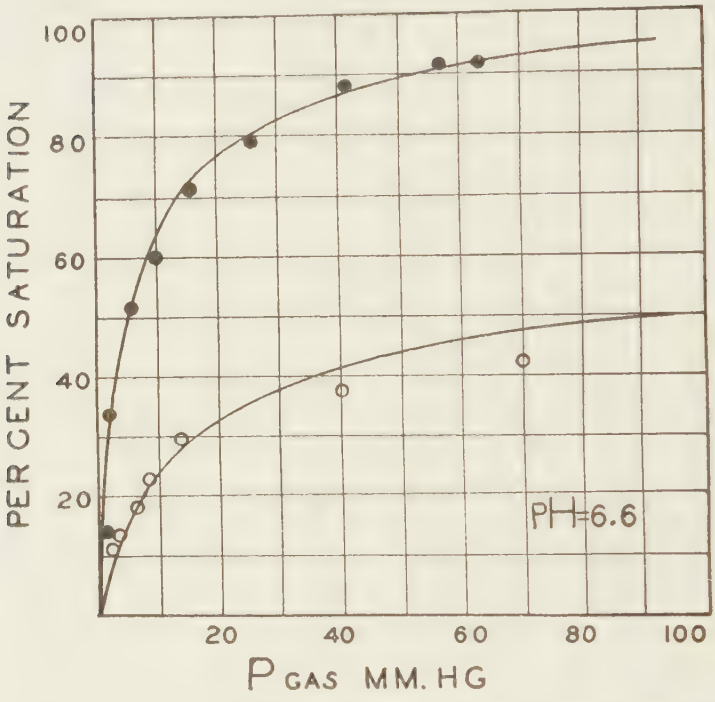

Fig. 2. The carbon monoxide (upper) and oxygen (lower) dissociation curves for sea-robin blood at pH 6.6. The oxygen pressure for each point on the oxygen curve has been divided by 10. The curves drawn through the points are rectangular hyperbolas with 50 per cent inactivation of hemoglobin assumed for the oxygen curve.

oxide dissociation curve is about 165 times greater than that for the oxygen dissociation curve. From the data it would seem that sea-robin blood at this pH behaves in a manner similar to human blood.

In more acid ranges the oxygen dissociation curves exhibit the apparent decrease in oxygen capacity described for several other

fish bloods. The carbon monoxide dissociation curves, on the other hand, at comparable pH values do not show this phenomenon to any appreciable extent. Furthermore, the two sets of curves are definitely different. For example, at pH 6.6, even though both curves can be satisfactorily described by rectangular hyperbolas,

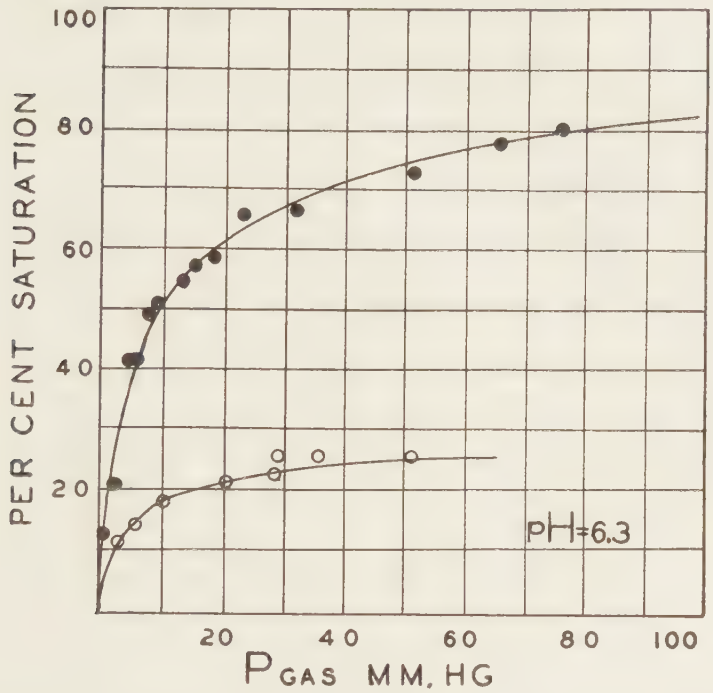

FIG. 3. The carbon monoxide (upper) and oxygen (lower) dissociation curves for sea-robin blood at pH 6.3. The oxygen pressure for each point on the oxygen curve has been divided by 10. A smooth curve not quite equal to a rectangular hyperbola passes through the data for the carbon monoxide dissociation. The lower curve is a rectangular hyperbola with 75 per cent inactivation of the hemoglobin assumed.

the oxygen curve can only be so described by assuming that the hemoglobin is 50 per cent inactivated. When the blood is made still more acid (pH 6.3), the carbon monoxide capacity of the hemoglobin is unchanged, whereas apparently only 25 per cent of the normal oxygen capacity remains. Even at pH 5.5 the blood is 85 or 90 per cent saturated with carbon monoxide at 500 mm.

pressure, and the shape of the curve is such as to suggest that the blood would become completely saturated if the carbon monoxide tension were made greater.

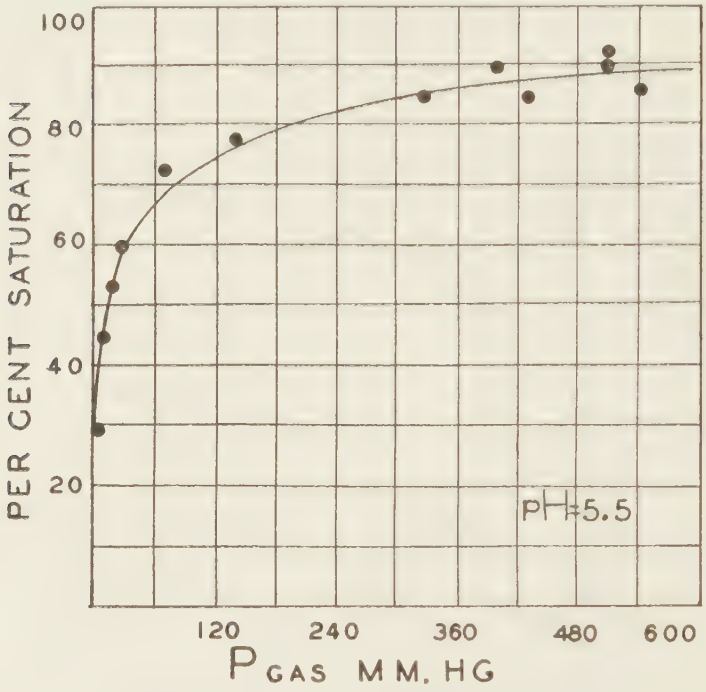

FIG. 4. The carbon monoxide curve for sea-robin blood at pH 5.5. A smooth curve not quite equal to a rectangular hyperbola passes through the points.

Carbon Monoxide Dissociation Curves for Toadfish and Tautog Bloods

Only one carbon monoxide curve was established for each of these bloods. The pH at which each curve was established is approximately 6.5, which is the acidity at which the oxygen dissociation curves indicate that approximately 50 per cent of the hemoglobin is inactivated (Green and Root, 1933). The data for the carbon monoxide dissociation curves are presented in Fig. 5. The position of the oxygen curves at the same pH is also shown in the figure for the sake of comparison.

By inspection of the carbon monoxide curves it becomes apparent that there is no reversible inactivation of the hemoglobins as far as carbon monoxide is concerned. A situation comparable to that described for sea-robin blood appears to be the case, for the

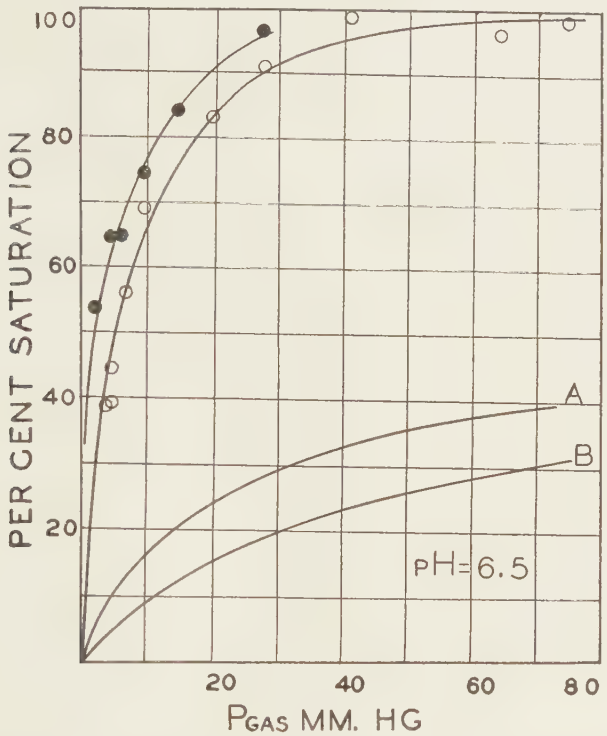

Fig. 5. The carbon monoxide dissociation curves for toadfish (upper, with dots) and tautog (lower, with circles) bloods at pH 6.5. The two lower curves represent the position of the corresponding oxygen dissociation curves, Curve A being that for toadfish, and Curve B that for tautog blood.

oxygen and carbon monoxide curves are quite different. In order to fit the oxygen curves with the usual equations it is necessary to assume inactivation, whereas this is obviously unnecessary in the case of the carbon monoxide dissociation curves.

We wish to express to Professor A. C. Redfield our sincere thanks for his helpful suggestions and criticisms. We also wish to thank Mr. Walter Buck for technical assistance.

SUMMARY

The behavior of fish hemoglobin toward carbon monoxide and oxygen has been compared. It differs in two respects.

1. The hemoglobin can be saturated with carbon monoxide regardless of the pH (within the limits set by the experiments), whereas at acid reactions oxygen apparently combines with only a portion of the hemoglobin present.

2. At acid reactions the dissociation curves at comparable pH values differ in shape.

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TEMPERATURE-SALINITY CORRELATION WITHIN THE FLORIDA CURRENT

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The observations of the Bache Expedition (1914) enabled Wüst (Florida- und Antillenstrom, Veroff. Inst. Meereskunde, N.F., Reihe A, Heft 12, pp. 1-48, Berlin, 1924) to publish a curve which gives the relationship between temperature and salinity for the waters flowing through the Straits of Florida. A careful comparison of this curve with observations from the Sargasso Sea shows that for any given temperature between 15° and 6° the Florida Current Water as it leaves the Straits is from 0.05 to 0.20 parts per thousand less saline than water from the Western North Atlantic in similar latitudes. These lower salinities are especially striking in the deeper parts of the Current. For example, Wüst's curve shows that 6°-water in the Straits of Florida has a salinity of about 34.84 parts per thousand, while from recent Atlantis observations we know that the same temperature in the Antilles Current or in the Sargasso Sea would give a salinity value of about 35.03 parts per thousand.

The explanation of this situation is not hard to find. From the Dana Expedition (J. P. Jacobsen, Contributions to the hydrography of the North Atlantic, The Dana Expedition 1921-22, pp. 1-98, Copenhagen, 1929) we know that the relatively fresh intermediate (700 to 1200) layer, which is the dominant feature of the whole South Atlantic, can be easily traced north of the equator for some distance. Wüst (Der Ursprung der Atlantischen Tiefenwasser, Jubilaums Sonderband, Zs. Ges. Erdkunde, pp. 506-534, 1928) found that in mid-ocean this fresh layer of antarctic origin extends to about latitude 20° north. Recent observations in the Caribbean Sea show that this southern water penetrates between the islands of the Lesser Antilles and spreads north and west towards the Yucatan Channel. It is understandable then that the colder waters of the outflow from the Straits of Florida should have relatively low salinities reflecting their origin from the Equatorial Current. Thus the current-system of the Caribbean and the Gulf of Mexico carries some of the antarctic layer considerably further north than it is able to spread in mid-ocean.

Since the deeper waters in the Straits of Florida can be distinguished by their relative freshness, it would be interesting to learn how far northward this remnant of antarctic water can be traced in the Florida Current. Three important sections, made by the Atlantis during May 1933, have provided us with ideal material for this study. The first of these crossed the current off Cape Canaveral, 75 miles north of the mouth

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1)Contribution No. 51 from the Woods Hole Oceanographic Institution.

of the Straits. The next ran eastward from Jacksonville, Florida, while the third was located off Onslow Bay, about 115 miles south of Cape Hatteras. Thus we can examine the average ratio of T to S at mid-depth in the heart of the Current at three points between the Bahama Banks and Cape Hatteras. The results are tabulated in Table 1.

Table 1--Salinity, S, in parts per thousand at different temperatures, T

Region	Temperature					
	6°	7°	8°	9°	10°	11°
Florida Straits (Wüst)	34.84	34.88	34.93	35.01	35.15	35.30
Cape Canaveral	.....	34.88	34.96	34.08	34.22	35.36
Jacksonville	.....	.....	35.02	35.12	35.23	35.38
Onslow Bay	35.04	35.06	35.12	35.14	35.22	35.36
Sargasso Sea (average)	35.03	35.07	35.12	35.20	35.29	35.41

Since off Cape Canaveral and Jacksonville the Current is flowing over the relatively shallow Pourtales Plateau, its depth is there limited to about 800 meters and water as cold as 6° does not appear in the sections. Thus we learn that the bottom-water from the deeper parts of the Straits of Florida is not able to flow northward and that after the Current has left the Pourtales Plateau (off Onslow Bay), typical Sargasso-Sea Water is found in the layers colder than 8°. But the water warmer than 8° still bears the stamp of its southern origin as it approaches Cape Hatteras.

We can conclude then that as the Florida Current flows northward along the continental slope, it is constantly increasing in salinity at mid-depths through the admixture of the more saline waters of the Sargasso Sea. But as the Current approaches Cape Hatteras, there still remains in the layer having temperatures of from 9° to 11° some traces of its South Atlantic origin.

Further studies of this question are now in progress and a full examination of the available observations from the heart of the Gulf Stream north of Cape Hatteras is now being made with a view of finding just how far the low salinities at mid-depths in the Caribbean can be traced. It is rather startling to discover that the Gulf Stream, so long considered as carrying tropical waters northward, really has at mid-depths a considerable admixture of water of antarctic origin.

Woods Hole Oceanographic Institution,
Woods Hole, Massachusetts

Contribution No. 53

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WASTING DISEASE OF *ZOSTERA* IN AMERICAN WATERS*

BY

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THE unprecedented widespread occurrence and devastating nature of the prevalent disease of the eel-grass, *Zostera marina*, have been viewed with considerable concern by those directly affected by its disappearance. To plant pathologists and marine biologists, the ecological changes following the total disappearance of the plant from our Atlantic coast[1] and from the western coast of Europe[2] have been a matter of special interest.

FIG 1. Diagram showing association of the *Labyrinthula*-like organism within leaf tissue of *Zostera marina*.

In the summer of 1933, with the return of sporadic growths near Woods Hole and at various points on the eastern coast[2,3], there was begun in these laboratories a search for an agent responsible for the spotting and darkening of the leaves that are symptomatic of the disease. A number of bacteria were isolated from diseased specimens but none of these demonstrated pathogenicity satisfactorily, although it has been claimed that bacteria are the causative agents[4,5,6]. Further, we have been unable

* Contribution No. 53, Woods Hole Oceanographic Institution and Journal Series Paper of the N.J. Agr. Exp. Station.

to observe the *Ophiobolus*-like fungi reported by other investigators as commonly occurring on the rhizomes and leaves of diseased plants[7,8]. Rather, all of the specimens that we have examined, taken from various points along the coast and secured locally, show the presence, within the air spaces and in cells of the diseased leaves, of thin-walled, tenuous, spindle-shaped organisms. Their detection in preserved tissue depends upon careful fixation and staining as they are but slightly refractile. They are very difficult to observe in the living tissue, though, it might be mentioned, this has been done.

From our superficial examination the organism appears to be a mycetozoan very similar to the *Labyrinthula* described long ago by Cienkowski (1876), as parasitic upon filamentous marine algæ[9]. The individual organism is spindle-shaped with terminal, often branching, tenuous pseudopods. The air spaces of infected leaves are filled with net-like aggregates of these bodies, the members of which are attached to each other by their filamentous pseudopods.

In zones about the discoloured areas they may occur in more loosely organised chains. Within the cell they bring about disorganisation of the chloroplasts and disintegration of the nucleus—the cell wall is not attacked except at the point of entrance of the delicate pseudopod (see Fig. 1).

The life-history of the organism has not yet been studied in great detail. Further, although it is almost universally present in freshly collected diseased leaves observed by us, we are not satisfied that it is the causative agent of the disease, though it is placed under suspicion as a possible factor. The necessary isolation and inoculation experiments are under way in our laboratories. Despite the unfinished nature of our work, we believe the association of the organism with the diseased condition as found by us to be of sufficient importance to merit the earliest possible attention of those interested in the disease.

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THE DIURNAL MIGRATION OF COPEPODS IN ST. GEORGES HARBOR, BERMUDA

GEORGE L. CLARKE

(*From the Woods Hole Oceanographic Institution*[1] *and the Laboratory of General Physiology, Harvard University*)

The variable nature of the diurnal migration of plankton has rendered difficult the task of discovering the importance of different environmental conditions in causing and controlling the phenomenon. Progress may be made, however, by comparing the behavior of the animals under different circumstances. In view of the data already obtained on the diurnal migration of copepods in the Gulf of Maine (Clarke,1933*b*, 1934), it appeared worthwhile to make similar observations in Bermuda waters, where different species of copepods and entirely different environmental conditions existed. The observations, made in St. Georges Harbor on January 23–24, 1934, must be regarded as approximations since only one (non-closing) net was used. The migration is of such magnitude, however, that there can be no doubt as to its general nature.

The net, kindly loaned to me by Dr. William Beebe, was of the international type. It was 50 cm. in diameter at the mouth and 3 m. long with very coarse netting at the upper end and silk bolting cloth at the middle (12 strands/cm.) and at the bottom (23 strands/cm.). A standard run of a little less than half a mile was laid out between two landmarks in St. Georges Harbor. The tows were made from the Laboratory launch run over this standard course at as nearly the same speed as possible, and always in the same direction, from west to east. Each plankton haul, therefore, was made through the same amount of water. The times required for the run ranged between 10 and 19 minutes, the variability being caused by changing wind conditions. In each set of observations, a haul was made at the surface first and this was followed as quickly as possible by a haul near the bottom. (In one case a third haul was made at a depth halfway to the bottom.) The depth of water was between 12.5 and 14 meters in this part of St. Georges harbor. For the deep haul the net was lowered until the weight, 2 meters below the net, struck the bottom. Then the launch was started and 2 meters more line was payed out. About 20 minutes elapsed between

[1] Contribution No. 54.

the surface and the deep hauls. The time stated in the table for each set is the midpoint between the times for the two hauls.

No attempt was made to study the hydrographic conditions in St. Georges Harbor in detail. The surface temperature was about 20° C. and since a tidal current flowed through the harbor, it is probable that the water was fairly well mixed and hence uniform. A surface haul and a deep haul were taken over the standard run with a diatom net (1 meter long, 30 cm. in diameter, netting 76 strands/cm.). The catches, very kindly analyzed for me by Dr. J. F. G. Wheeler, contained the following total numbers of organisms:

	Surface	Deep
Detritus	Most abundant	Most abundant but less than at surface
Chætoceras (chains)	19,000	27,000
Rhizosolenia	93,000	90,000
Flagellate A. (peridinian?)	79,000	131,000
14 other small forms	24,000	22,000

The analysis of the zoöplankton hauls—carried out by counting and identifying under the microscope either the whole catch or a sample of it—showed the presence of only two species of copepods: *Calanopia americana* (Dahl) and *Acartia spinata* (Esterly). These were divided into adult males and females. Immature specimens were encountered in some of the hauls but were too few to be treated quantitatively. *Calanopia* was found to be the dominant copepod in the bays and harbors of Bermuda at this time (*cf.* Esterly, 1911). It is true that this species was taken in the outside waters but in rapidly diminishing numbers with increasing distance from the mouths of the inland waterways. *Acartia,* on the other hand, was more numerous in the offshore waters and appeared to have been swept into the harbors by the tide.

In the diurnal migration of *Calanopia* and of *Acartia* (Table I) the males and females behaved in the same way. At mid-day the *Calanopia* population could not be found at any depth in the harbor. The animals were undoubtedly so close to the bottom at this time that they could not be caught by the plankton net. In fact, the copepods probably actually burrowed into the soft mud bottom, as is discussed below. At 1944 (local sun time) *Calanopia* was taken in large numbers in the surface hauls and in still larger numbers in the deep hauls. Early the next morning, while it was still dark, the relative distribution of the population was unchanged although all the catches were slightly smaller. Just before sunrise, the numbers of *Calanopia* at both levels were greatly reduced. Although no hauls were taken at noon on the second day, the water was undoubtedly entirely deserted just as before, and at 1707, a

quarter of an hour before sunset, the beginnings of the upward migration are apparent. Less than an hour later the numbers of *Calanopia* taken in the surface hauls had increased 10-fold and the numbers taken in the deep hauls had increased 100-fold.

TABLE I

Diurnal migration in St. Georges Harbor, Bermuda. Total numbers of copepods.

	Wind	*Sky*	*Sunrise*	*Sunset*
Jan. 23, 1934.....	SE. moderate	Many clouds	—	1724
Jan. 24, 1934.....	W. slight to strong	Clear	0700	1725

	Local Sun Time	Jan. 23, 1934		Jan. 24, 1934			
		1054	1944	0511	0656	1707	1801
Calanopia americana male	Surface.....	0	530	330	15	2	20
	Intermediate.....			2180			
	Deep.....	0	2750	2110	16	17	2340
Calanopia americana female	Surface.....	0	740	510	13	3	50
	Intermediate.....			2370			
	Deep.....	1	4380	2370	12	27	2620
Acartia spinata male	Surface.....	0	90	50	1	0	0
	Intermediate.....			60			
	Deep.....	30	30	90	120	1	4
Acartia spinata female	Surface.....	0	230	60	2	1	2
	Intermediate.....			130			
	Deep.....	40	50	140	180	2	4

The vertical migration of *Acartia* followed the general plan of the *Calanopia* migration but the differences between the day and night hauls are not as striking. At mid-day a considerable number of *Acartia* were taken by the deep net, indicating that this species did not descend to the bottom as completely as did *Calanopia.* At 1944 the upward migration had brought the majority of the animals to the surface. The first set of hauls on the following morning showed a downward movement and this had been greatly accelerated by sunrise. *Acartia* was scarce at 1707 at both levels and even at 1801 no considerable numbers had appeared. This disappearance of the *Acartia* population was coincident with the rising of a strong northwest wind which may have caused a removal of this species, which did not descend entirely to the bottom, through the harbor mouth to the east. On January 23 and on the preceding days, a very strong easterly wind had been blowing. It appears possible, then, that at the beginning of the investigation on diurnal migration there

may have been more of this oceanic species present in the harbor than normal, and that during the second day these copepods were being carried back to the outside waters.

Unfortunately it was not possible to make light intensity measurements, but it is clear that the diurnal migration of both *Calanopia* and *Acartia* shows a close correspondence with the rising and setting of the sun. The rapidity of the vertical movements of these copepods is in accord with the shortness of the duration of twilight in low latitudes. The clearness of the water in this part of the ocean (*cf.* Clarke, 1933*a*), coupled with the brilliance of the sun, must have resulted in an intensity of irradiation at all depths in St. Georges Harbor far greater than exists in the surface layers of the Gulf of Maine. It would not be surprising then to find that *Calanopia* had become adapted actually to burrow into the mud during the middle of the day. In fact, during culturing experiments in the laboratory it was found that when individuals of this species were introduced into small glass dishes of sea water with a layer of harbor mud on the bottom, they buried themselves almost immediately. Other species of calanoid copepods did not exhibit this behavior. The animals undoubtedly leave the mud at night for the purpose of feeding (*cf.* Worthington, 1931; Nicholls, 1933). This upward movement might appear superficially similar to the diurnal migrations of certain bottom-living animals (*cf.* Fage, 1933), but Dr. C. B. Wilson informs me that there is every indication that *Calanopia* is a truly pelagic form. As we have seen above, food organisms were present in the water of the harbor, although here as in other warm regions phytoplankton was generally scarce.

To sum up, the copepods *Calanopia americana* and *Acartia spinata* were found to exist in St. Georges Harbor in large numbers near the surface and in the deeper strata at night and to migrate to the bottom at sunrise, the former species apparently burrowing into the mud. As in the Gulf of Maine, the diurnal migration appears to be correlated primarily with changes in light intensity during the course of the day, although it may be partly under the control of other environmental factors as well. The fact that the copepods descend to the bottom is consistent with the great intensity of the submarine irradiation in the Bermuda region.

I am indebted to the Bermuda Biological Station for the use of the facilities of the laboratory and I wish to thank the Director, Dr. J. F. G. Wheeler, for his helpful coöperation during the course of this investigation.

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THE CHEMICAL NATURE OF THE PIGMENTS AND THE TRANSFORMATIONS RESPONSIBLE FOR COLOR CHANGES IN PALÆMONETES

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I. The Chemical Nature of the Pigments

The most prevalent pigment of *Palæmonetes vulgaris* is a reddish one which in the living shrimp varies in tint from a clear red to a brownish black, and in consistency from a homogeneous, viscous liquid to a granular gel. The yellow pigment, like the red, is always located within definite chromatophores. This pigment is a transparent, homogeneous liquid less viscous than the red. The yellow and red pigments, though most commonly present in the same chromatophore center, always retain their individuality as two distinct masses of pigment.

Lönnberg and Hellström (1932) studied spectroscopically the two pigments that they found in the lobster. In distribution between petroleum ether and methyl alcohol a yellow pigment remained in the petroleum ether (absorption bands in petroleum ether, 490 and 457 mμ) and a reddish pigment went into the alcohol layer (absorption band in ether, 494 mμ). Kuhn and Lederer (1933) have worked upon the brownish black pigment of the Norwegian lobster, *Astacus gammarus,* and have determined it to have the chemical formula $C_{27}H_{32}O_3$ and to this they gave the name astacin. The absorption band of astacin in pyridin was found to be very broad and to have a maximum at 500 mμ. These men also found in their extracts a small amount of a substance which they admitted was probably carotin. Later, Fabre and Lederer (1934) showed that astacin was present in a number of other crustaceans. Very recently Karrer and Loewe (1934) have concluded that astacin was a derivative of β carotin.

A study has been made of the absorption spectra of the pigments of *Palæmonetes.* Shrimps were boiled to convert the blue pigment to red, then dried and extracted with chloroform. The resulting extract was orange-red in color. It was quite photosensitive, bleaching to a pale yellow during a few hours exposure to sunlight. Figure 1 shows the absorption spectrum of the pigment freshly extracted in chloroform. There is a single asymmetrical band with a maximum at about 480 mμ.

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This band is roughly the same shape as that determined by Redfield (1930), who examined a chloroform extract of the blood of the lobster, *Homarus*. The absorption spectrum of the lobster blood extract is also shown in Fig. 1.

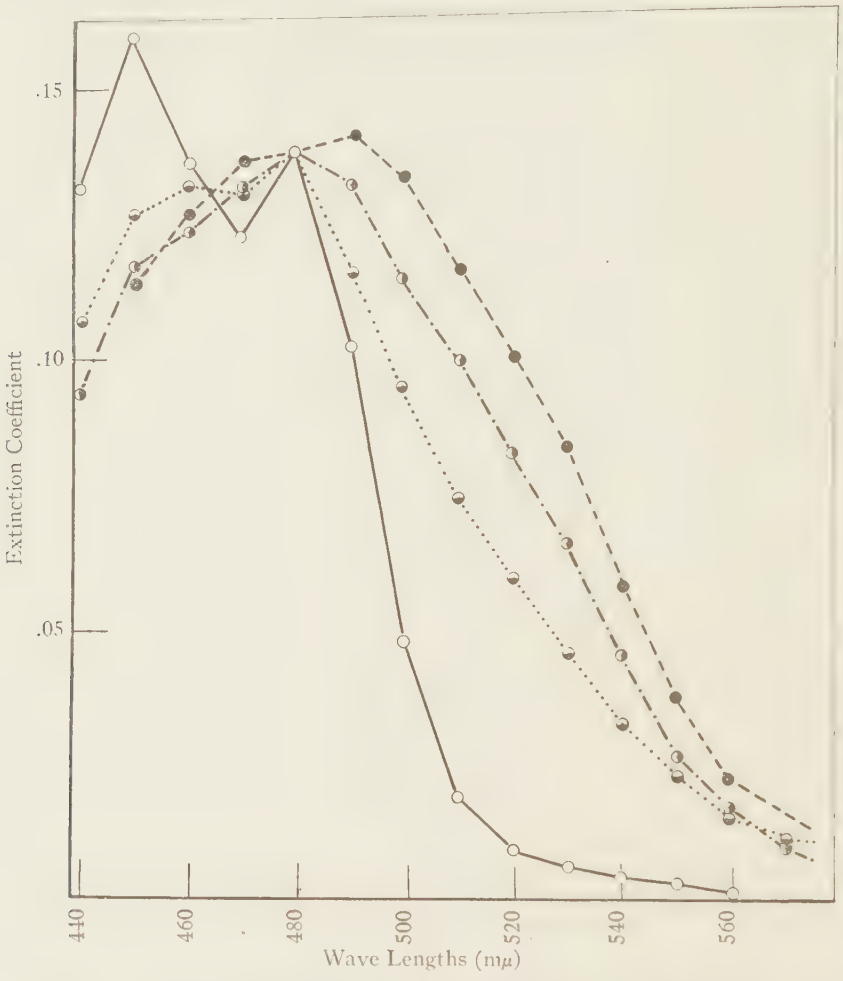

FIG. 1. The unbroken curve shows absorption spectrum of shrimp yellow pigment; – – – – shows absorption band of extract of lobster blood (Redfield, 1930); ····· shows absorption spectrum of extract of shrimps which have little red pigment in chromatophores; and —·—·— shows the absorption band of extract of shrimp which have much red. All measurements are made of pigments in chloroform solution.

Kuhn and Lederer have found that when a petroleum ether solution of astacin is shaken with a solution of sodium hydroxide in 90 per cent

methyl alcohol the astacin enters the methyl alcohol layer as a sodium salt of astacin. When this layer is acidified the astacin comes out of solution as violet crystals. It has been found that if the pigment obtained by extraction of the whole *Palæmonetes* with chloroform was

FIG. 2. The unbroken line represents the absorption bands of shrimp yellow pigment in chloroform; – – – – shows the absorption bands of plant carotin in alcohol and ether as measured by McNicholas (1931).

dissolved in petroleum ether and then shaken with a solution of potassium hydroxide in 90 per cent methyl alcohol, a reddish pigment entered the methyl alcohol layer and a yellow pigment remained in the petroleum ether. Figure 2 shows the absorption spectrum of the petro-

leum ether component after it had been redissolved in chloroform. Plotted upon the same coördinates is the absorption spectrum of a solution of plant carotin in alcohol and ether as it was measured by McNicholas (1931). The shrimp pigment strikingly resembles plant carotin. Living *Palæmonetes* contain a greater proportion of yellow pigment than do lobsters and correspondingly a greater percentage of this fraction was obtained than was found by Kuhn and Lederer.

Samples of shrimp differing from one another in the relative proportions of red and yellow pigments visible in their chromatophores showed absorption spectra differing from one another in the extent to which the band was shifted in one direction or the other. The limits of this shift were defined by the absorption spectrum of carotin on the one hand and that of the lobster red pigment on the other. Figure 1 shows the absorption spectra of the whole pigment from two lots of shrimp. In one lot much red pigment was present in the chromatophores, and in the other there was considerably less. It thus seems probable that these differences are explained by the varying percentages of astacin and carotin in the extracts.

These results strongly suggest that the pigment of the yellow chromatophores of *Palæmonetes vulgaris* is carotin, $C_{40}H_{56}$, and that the pigment of the red chromatophores is one or another compound of astacin.

II. Chemical and Morphological Transformations Responsible for Color Changes

Thus far, nearly all the work upon color changes in crustaceans has been done upon the rapid responses of the chromatophore as indicated by the migration of pigment within the pigment cells. In reality, the well-adapted shrimps and prawns in nature owe their adaptations at least as much to the differential formation and destruction of pigments within the chromatophores as to the more rapid migration of the already formed pigments. Although this fact has been noted by the majority of workers in the field, little actual research has been devoted to it.

The following experiments were carried out upon adult *Palæmonetes vulgaris,* and were designed primarily to show the rate at which the pigmentation of the shrimps could be changed as a result of light, darkness, and black and white backgrounds.

Observations on Destruction of Red and Yellow Pigments

A shrimp was taken from a black background and placed upon white. In 20 minutes the red and yellow pigments had concentrated

considerably. A blue network was left behind in the region that was formerly covered by the red and yellow pigments. The concentrated yellow was a clear and homogeneous pigment but the red contained much of the blue pigment mixed with it, making it purplish red and chocolate in color.

In about six hours the blue network had gradually faded and the yellow and brownish pigments had completely concentrated.

For the following six or eight days there were faint bluish processes extending from the brownish pigment. The blue pigment seemed to be diffusing away from this chromatophore and at the same time the chromatophore was becoming more and more reddish.

At the end of eleven days there was practically no blue pigment left in the red, but clustered around the red were many small blue triangular crystals.

After fifteen days the red pigment had become homogeneous and had decreased to only a small fraction of its original amount. Globules of red pigment had left the chromatophore and were in the tissues around it.

At the end of three weeks nothing was left of the red pigment except small droplets scattered about. The yellow chromatophore was still intact and functional but had lost a large quantity of its pigment. Observations were continued for two more weeks. The yellow pigment continued to decrease in amount but was still present at the end of this time.

Plate I, Fig. 7, shows a red, yellow, and blue chromatophore after two days upon a white background, and Fig. 8 shows the same region of the same shrimp after sixteen days upon the same background. Notice the small droplets of red pigment that still persist. The time required for the complete disappearance of the red and blue pigments is a function of the quantities present at the beginning of the experiment.

Observations on Formation of Red and Yellow Pigments

An experiment upon formation of pigment was begun by selecting a shrimp which had been kept upon a white background for five weeks and then seeking a chromatophore region where only yellow pigment remained. A photomicrograph (Fig. 9) was taken of the region and then the shrimp was placed in a salt water circulating apparatus upon a black background. At the end of five days the region was again examined. The yellow pigment was broadly dispersed and had assumed a pale orange tint. Some new red pigment had commenced to form. This last was surrounded by the characteristic blue network. The shrimp was placed upon a white background and after an hour the

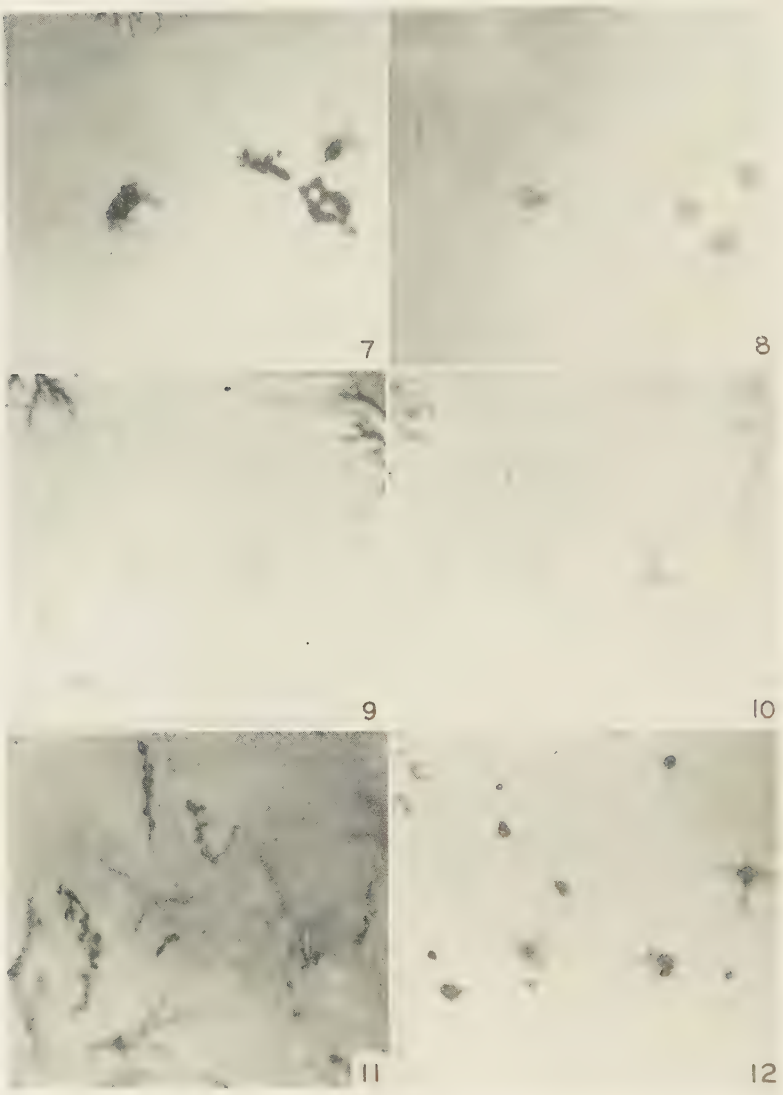

PLATE I

FIG. 7. Red and yellow chromatophores of a shrimp which has been two days upon a white background.

FIG. 8. Same area as in the preceding figure but photographed after two weeks upon a white background.

FIG. 9. Some chromatophores in a shrimp that has been five weeks upon a white background. Only yellow pigment is present.

FIG. 10. Same area as in Fig. 9, and showing the formation of red pigment after five days upon a black background.

FIG. 11. Same area as in Fig. 10, but photographed after two weeks upon a black background. Much red pigment has formed and a blue network is visible.

FIG. 12. Same area as in Fig. 11, but the animal has been one day upon a white background.

region was again photographed (Fig. 10). After two weeks upon a black background the shrimp was becoming quite dark in color. Much red had formed and the blue network was heavy. After an hour upon white the chromatophores of the experimental region were photographed (Fig. 11). This was repeated after 24 hours upon white (Fig. 12) in order to determine definitely whether new chromatophore centers had actually been formed. It was unquestionable that new red and blue pigments had formed where there was not a visible trace of a chromatophore at the beginning. Thus it is reasonable to suppose that red and blue pigments may arise quite independently of the yellow. The original yellow pigment now had a reddish tint instead of its former lemon yellow.

The blue network which gradually faded out during the 24 hours upon the white background reappeared during the next three days upon a white background, but now, instead of being a continuous network the blue pigment appeared in clumps around the red masses of pigment. This blue remained for two weeks in spite of the white background.

In this experiment the red appeared before the blue and the latter seemed always associated with the former pigment rather than with the yellow.

Formation and Destruction of White Pigment

A shrimp that was kept upon a white background for nearly two months had such a quantity of white pigment in its chromatophores that when this was dispersed it covered a considerable portion of the body. Due to the transparency of *Palæmonetes* white shrimps were ordinarily quite well concealed even upon a black background. However, when the white pigment had increased in quantity to the extent that it had in this instance, the shrimp stood out in sharp contrast when placed in a black dish. The stomach, due to the mass of white chromatophores upon it and its muscular contractions, fairly sparkled. Even after this shrimp had been upon a black background long enough for the white pigment to concentrate, the shrimp was still studded with clearly visible white spots. This was not altogether due to the quantity of white pigment present, but also to the absence of the red and blue pigments which would ordinarily assist in concealing it in such circumstances. It is such an example as this that makes me disagree with those workers in the past who have asserted that the white chromatophores play no part in color adaptation.

I have made no record of changes within an individual shrimp, but the general tendency of more and larger white chromatophores when a shrimp was long upon a white background, and fewer and smaller white

chromatophores in shrimps long upon a black background was striking enough to warrant this note of the fact.

Destruction of Blue Pigment

Although the origin of diffused blue pigment of *Palæmonetes* seemed to be the red pigment and the manner of its arrival in the tissues around the chromatophores and throughout the body seemed to be one of simple spreading, yet the disappearance of the pigment from the tissues appeared to be a question of destruction *in situ*. There has been no evidence that once the blue pigment was outside of the red pigment, there was any controlled migration back into it. An experiment was carried out to determine the effects of a white background and darkness upon the rate of destruction of the blue pigment.

Twenty-four deep blue shrimps were taken from a blue background and divided into two lots of twelve shrimps each. One of these lots was placed upon white background in the full light of a north window. The other lot was placed in total darkness. At the end of six hours the shrimps kept in the light had lost their blue coloration completely. In general appearance they were a yellowish white. The shrimps in darkness were promptly examined in a white light and found to be just as blue as at the moment when they were placed in the dark, but during the following six days there was a gradual disappearance of the blue network and the blue tint of all the tissues of the shrimps. Although blue was destroyed both in darkness and upon a white background in the light, the white background called forth a much more rapid destruction than did darkness.

A Quantitative Study of the Chemical Transformations in the Pigments

Hitherto no attempt has been made, as far as I am aware, to work out quantitatively the rates of pigment formation and destruction in crustaceans. A method is here set forth whereby quantitative determinations of the relative amounts of pigment in lots of shrimp subjected to various conditions can be made. The method is subject to some error due to the instability of the pigment itself, but since the results are relative and the technic uniform, it yields on the whole quite serviceable data. It gives one value which is the sum of the red, yellow, and blue pigments. The first step in the process is to convert the blue pigment to red by immersing the shrimps for a minute in boiling water. The work of Newbigin (1897) indicates that the red pigment that results from the heating of the blue is probably the same as the red that is present in the living shrimp.

The following is the technic that has been used in these experiments. Shrimps to be tested were dipped into boiling water for one to two minutes and then removed, cut into small pieces, and placed in a vacuum desiccator in darkness. At the end of 48 hours drying was completed and the dried shrimps were carefully weighed and then extracted with chloroform in the proportions of 30 cc. of chloroform to 1 gram dry weight of shrimps. The extraction was allowed to proceed for 24 hours, during which time the mixture was kept in a tightly stoppered test-tube in a refrigerator. After this interval of time the material was so well extracted that the remainder could be neglected. What would have been gained by a longer time of extraction would have been lost by the bleaching of the pigment. In the presence of light and air the bleaching occurred quite rapidly. Another source of error lay in the fact that the solvent was very volatile and thus the solution tended to concentrate in the course of the manipulations. Because of the volatility and photochemical activity of the material, the precautions taken during the drying and extracting were important ones.

At the end of 24 hours of extraction the solution was rapidly filtered and then the concentration of the pigment compared with that of a standard solution by the use of a colorimeter. Beer's law states that the amount of light transmitted by a solution varies inversely with the concentration of the absorbing substance. Hence a value for the concentration of the pigment in terms of the standard could be readily calculated. The standard solution was made by adding 1 cc. of methyl orange (.02 per cent in aqueous solution) to 16 cc. of an acetic acid sodium acetate buffer solution having a pH of 4.

One difficulty experienced in this method of obtaining values for the concentration was that different lots of shrimps contained different proportions of red and yellow pigments. This caused the solutions of pigment to differ from one another in tint. Considering that the colorimeter required the solutions compared to be the same color, some inaccuracy was introduced on this score. In order to determine the extent of this error several of the experiments were made and both the colorimeter and a spectrophotometer were used to measure the relative absorption of light by the solutions. The latter instrument was not affected by the color of the solution for here the light of only a definite wave-length was measured. The wave-length selected was 480 mμ, the peak of the absorption spectrum of the pigment of these crustaceans. The results obtained by the two instruments checked each other very well. For most of the experiments the colorimeter was used alone because of the rapidity of operation.

When the effect of a given set of conditions upon the amount of

pigment in the shrimp was to be determined, a large number of shrimps were placed in the desired situation. At the beginning of the experiment and at three- or four-day intervals thereafter about twenty shrimps were taken from the lot as a sample to test for the concentration of the pigments. The value obtained was taken as a criterion of the concentration of pigment in the whole lot of shrimps at that particular moment. As this method rested to a great extent upon a statistical

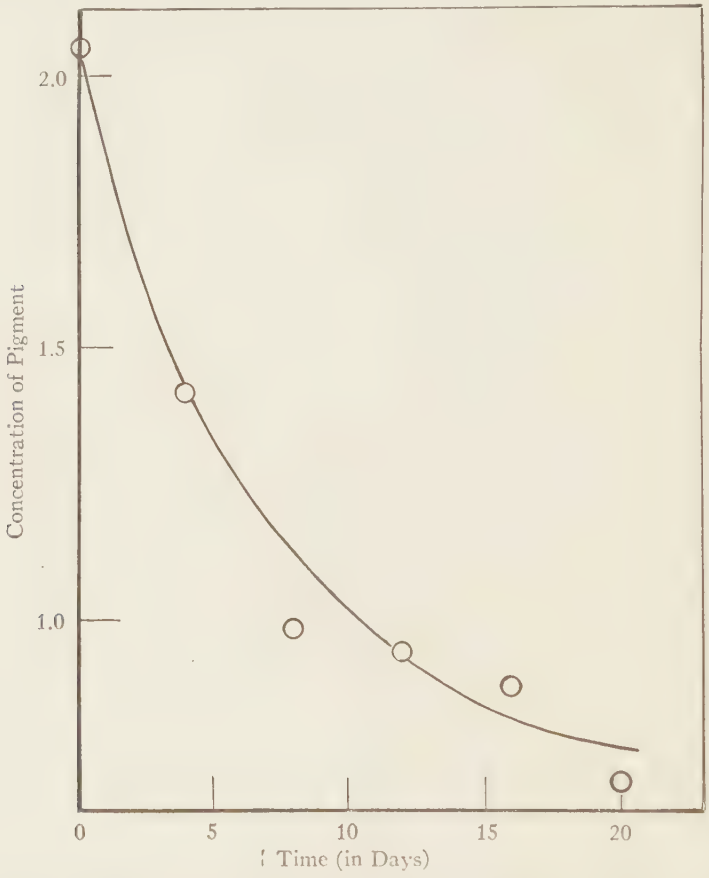

FIG. 3. Decrease in concentration of pigment in shrimps which were kept upon a white background in light. Concentration is stated in terms of a methyl orange standard solution.

basis, much of the irregularity of the results was attributed to the fact that so few shrimps were taken in each sample. There is little doubt that had the samples consisted of as many as fifty shrimps, the data exhibiting the effects of the environment upon the pigment content of the shrimps would have more closely fit a smooth curve when plotted.

Pigment Destruction upon a White Background

Approximately 250 shrimps were placed in a large white enamelled tub through which sea water was flowing. The tub was located in the center of a small room lighted by large north windows. During the course of the experiment the animals were fed upon finely chopped *Modiolus* and *Mytilus*. This experiment was repeated five times during the summer of 1932 with strikingly similar results. Figure 3 is typical of these experiments.

An experiment carried on in a similar manner except that the shrimps were starved over the period of twenty-four days yielded results showing the same rate of destruction of the pigments.

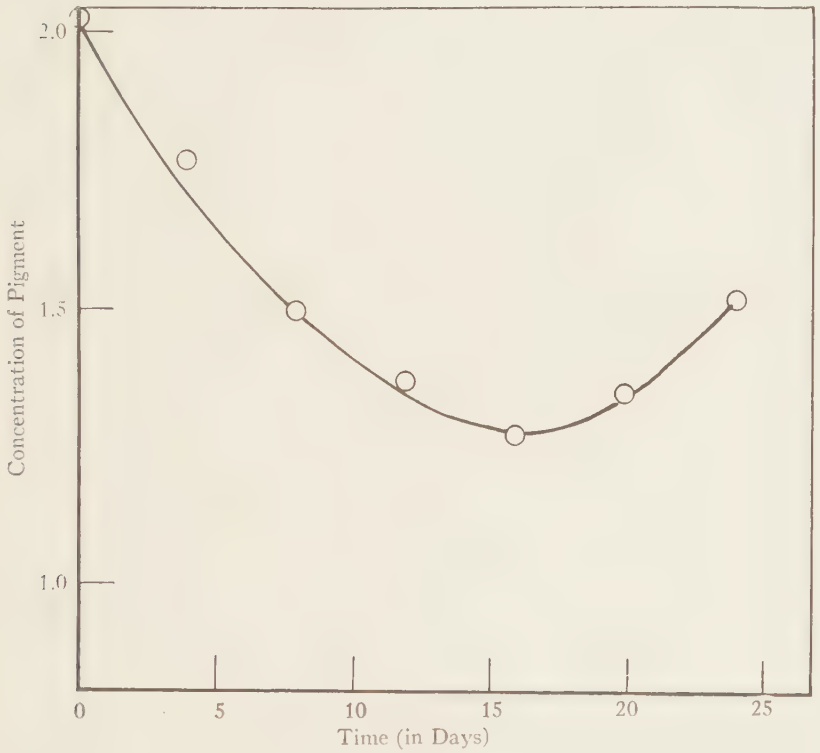

FIG. 4. Change in concentration of pigment in shrimps which were kept in darkness. Concentration is stated in terms of a methyl orange standard solution.

Effect of Darkness upon Pigment Quantity

In a second type of experiment shrimps were placed in large containers and allowed to remain in total darkness except for the brief moments when samples were being taken and the animals were being

fed. At these times a weak red light was turned on. This experiment was repeated six times during the summer. Sometimes the shrimps were fed and at other times starved during the course of the experiment. Again there was no difference between the results obtained when the shrimps were fed and when they were starved. This can be explained by the cannibalistic nature of the shrimps. Figure 4 is typical of the results.

The change from pigment decrease to pigment increase at the end of about fourteen days was constant in all the experiments and was quite curious. It does not agree with the observations of Brooks and Herrick (1891). These men found that *Palæmonetes vulgaris* was bleached nearly white after eighteen days in darkness. Their animals were sealed up during the entire period whereas mine were subjected to the occasional stimulation of the red light. This question will bear further investigation as it perhaps holds an explanation as to why deep sea crustaceans are generally heavily pigmented with red. In the deep sea it is known that the conditions are those of nearly total darkness. On the other hand, crustaceans which inhabit underground caves where there is an absence of light are usually conspicuous by their lack of pigment.

Pigment Formation upon a Black Background

In a third experiment a batch of partially bleached shrimps was put into large crystallizing dishes the outsides of which were painted with flat black paint. The dishes were left in the daylight of the laboratory. Figure 5 gives an example of the type of result that was obtained in four different experiments.

Control Experiment

As a control experiment for the preceding ones, a large glass aquarium was filled with sea water and in the aquarium was placed an abundance of the alga, *Fucus,* from which these shrimps were taken in nature. As nearly as could be judged, this situation very closely simulated the normal habitat of the shrimps both in color of the environment and in light intensity. The results taken from shrimps kept in this aquarium are seen in Fig. 6.

Discussion of Results

From the graphs of the experiments just described the following facts are evident. Upon a white background in the light there is a very rapid destruction of pigment within the shrimps. This destruction is continued over the twenty-day period of the experiment. In

another trial of the same nature the pigment was continuing to disappear even after thirty days. In this recorded case the pigment concentration decreased 65 per cent in the twenty days.

In darkness the rate of bleaching of the animal was considerably less than that seen upon a white background. This destruction of pig-

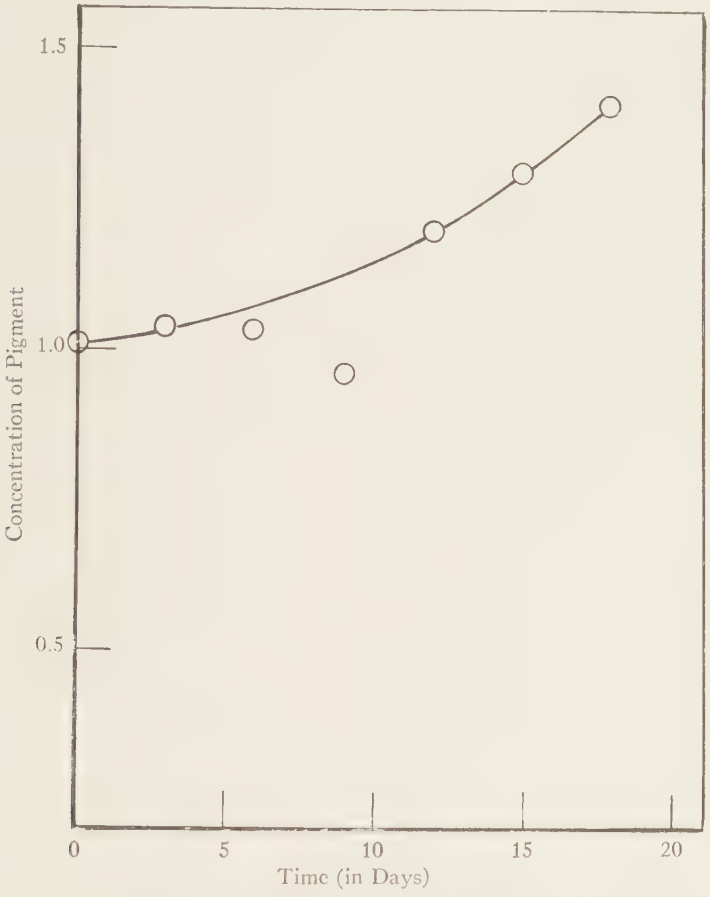

FIG. 5. Increase in concentration of pigment in shrimps which were kept upon a black background in light. Concentration is stated in terms of a methyl orange standard solution.

ment continued at approximately the same rate for sixteen days and then there was a reversal to pigment formation. In various experiments the time of this reversal varied from eleven to sixteen days after the animals were placed in darkness.

In the case of the partially bleached shrimps that were placed upon

a black background there was at first only a slow formation of pigment and then an increase in rate. The rate continued to increase over the eighteen-day period of the experiment. During this time there was a 40 per cent increase in the amount of pigment in the shrimps.

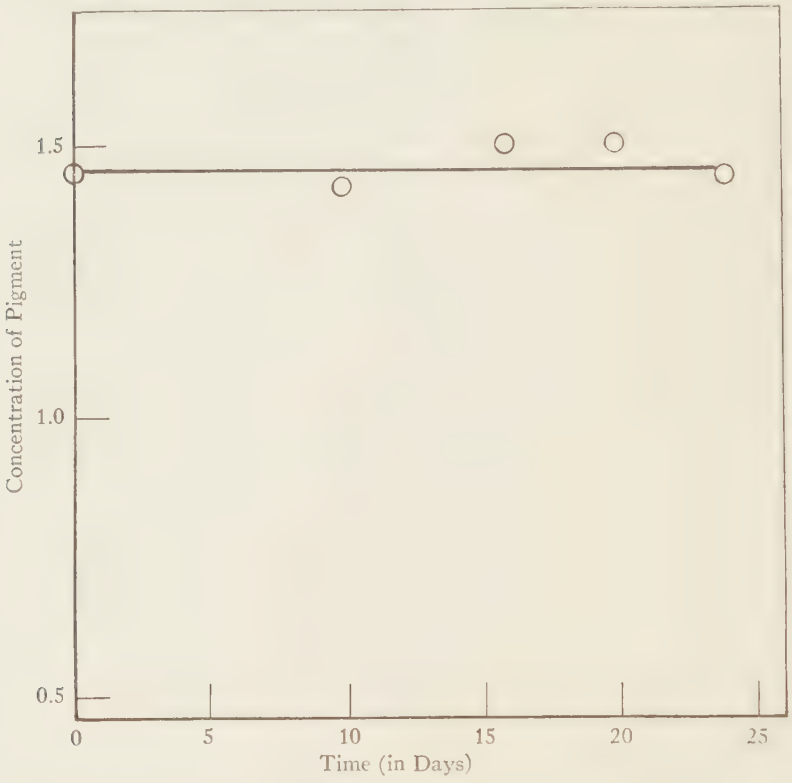

FIG. 6. Condition of concentration of pigment in shrimps which were kept in an environment which closely simulated the normal habitat. Concentration is in terms of a methyl orange standard solution.

The validity of this experimental technic was again checked in observing the behavior of the concentration of pigment of shrimps that were kept in apparently normal conditions. Here, over a period of twenty-four days, an interval of time as great as that of the longest experiment, there was neither an increase nor a decrease in the concentration of pigment.

It was shown by separating the extracted pigment of the animals into its two components by shaking a petroleum ether solution of the pigment with a strong solution of potassium hydroxide in 90 per cent methyl alcohol, that the shapes of these curves illustrating the rates of

formation and destruction of pigment were almost wholly effected by the increase and decrease in the amounts of the red and blue. The rate of change of the quantity of the yellow pigment was relatively insignificant.

Controlling Mechanism of Pigment Formation and Destruction

Keeble and Gamble (1904), who were at the time working upon the prawn, *Hippolyte varians,* put forth an hypothesis to account for pigment formation and destruction. Since that time this hypothesis has been restated by other investigators such as Babák (1913), and Odiorne (1933). In substance it states that a dispersed pigment is increasing in amount while a concentrated one is decreasing.

The observations in this report confirm the hypothesis originated by Keeble and Gamble. For example, on a white background there is a strong concentration of the red and yellow pigments, an absence of diffusing blue, and a strong dispersion of the reflecting white pigment. Correspondingly there is an increase in the amount of white pigment and a decrease in the amounts of the other three pigments. A black background, just as the hypothesis calls for, brings about an increase in the quantities of the red, yellow, and blue pigments and at the same time a decrease in the amount of white pigment within the chromatophores. Darkness causes a destruction of red pigment at about half the rate seen in an animal upon a white background in the light and here the red pigment is slightly dispersed.

On the basis of these facts it is possible that pigment formation and destruction in adaptation to colored backgrounds may be controlled by the same humoral agents that are responsible for the control of migration of the pigments within the chromatophores.

I take this opportunity to acknowledge my indebtedness to Dr. G. H. Parker and Dr. A. C. Redfield for their invaluable criticisms and suggestions.

Summary

The red pigment of *Palæmonetes vulgaris* appears to be astacin, while the yellow seems to be identical with plant carotin.

A method is set forth to measure the rate of formation and destruction of pigment within the shrimps. It is observed that the red and blue pigments are most rapidly formed and destroyed, while the yellow and white pigments are much more slowly changed.

Upon a white background in the light the red and blue pigments are destroyed very rapidly. Yellow disappears slowly.

Upon a black background in the light the red and blue pigments are formed.

Shrimps kept in darkness at first lose through destruction their red and blue pigment but after about two weeks there occurs a reversal of this process (pigment formation).

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Contribution No. 56

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DIGESTIVE ENZYMES OF THE PELAGIC COPEPOD, CALANUS FINMARCHICUS

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Yonge (1931), in his review of the "Digestive Processes in Marine Invertebrates and Fishes" states that "Little is known of the digestive enzymes of the Entomostraca. . . ." The somewhat incomplete observations here presented are sufficiently illuminating (I believe) to merit publication at this time, particularly since circumstances force me to postpone, for an indefinite period, carrying the work any further.

The feeding mechanism of the calanoid copepods has been described by Storch and Pfisterer (1925), and, perhaps more correctly, by Cannon (1928). They are filter feeders, and their mechanism is so efficient, according to Naumann (1923), that they can remove particles as small as 1 μ in diameter from the water. They can also ingest much larger particles, breaking up and swallowing large diatoms and crustacean nauplii, etc. (Cf. Dakin (1908), Esterly (1916), Lebour (1922), and Marshall (1924) for material observed in the gut.)

Calanus is so small (2.5–6.5 mm.) that it proved impractical to extract the guts alone. Large numbers of the live copepods (copepodid III to adult stages—mostly copepodid IV and V) were separated from other organisms taken in the tows, strained off on bolting silk, and placed in a calibrated test-tube. To each cubic centimeter of copepods was added 0.6 cc. of sea water, 0.4 cc. of 95 per cent alcohol, 1 cc. of toluene, and some glass beads or well washed sand. The tube was stoppered with a rubber stopper (previously boiled in a solution of NaOH and well washed) and shaken violently in a mechanical shaker for an hour or more, until the copepods were reduced to a pink emulsoid. The extraction was then allowed to continue for about 36 hours, and the extract filtered off through filter paper. More toluene was added, and the extract kept at 5–6° C. till used (1 to 7 days). Before use the extract was mixed with an equal volume of buffer. The buffers were made according to the directions given in Clark (1928). Those of pH 1.0, 1.5, and 2.0 were HCl-KCl buffers. For pH 2.4, 2.8, 3.2, 2.6, 4.0, 4.4, 4.8, 5.2, 5.6, and 6.0 the phthalate series was used. For pH 6.4, 6.8, 7.0, 7.2, 7.6, and 8.0 the phosphate series was employed; and for pH 8.49, 8.84, 9.61, 10.96, 11.45, and 12.54 the glycine series.

[1] Contribution No. 56.

These buffers were used in preference to others because it was known that they have no toxic effect on enzymes.

Since the extract itself was at pH 7.0–7.6, and since it is fairly well buffered, it is probable that the pH of the extract-buffer mixtures in the acid range was actually somewhat higher, and those in the alkaline range somewhat lower than the figures given.

No method was devised for accurately determining the pH of the gut of the living copepods. Neutral red colors the lumen so slightly and the gut is so narrow, that it is impossible, without adequate standards, to be certain that the color observed in the mid-gut was actually (as it appeared) the yellowish red of a somewhat alkaline solution, rather than very dilute brick or rose red of neutral or acid conditions. In the decapods (according to the data summarized by Yonge), for which the stomach pH is known, the normal conditions are acid (5.1–5.6), but it would be unsafe to generalize about other Crustacea from this. It may be said that there is nothing known about gut conditions in general, nor about the enzymes of *Calanus* (as will be seen below) to indicate that the gut pH might not theoretically lie anywhere in a range of from at least as low as 6.0 to at least as high as 8.0.

In the following experiments controls, using buffer alone, and buffer with extract kept 10 minutes in a tube in a boiling water bath, were used except where otherwise noted. The four lots of extract used were checked against each other and found to vary somewhat in strength, but were otherwise similar. Each observation given is based on at least two independent experiments—usually three to six.

Proteinase

The method used was that of Pickford and Dorris (1934). Digestion was found to occur from pH 2.4 to 12.54. Above pH 10.96 there was some alkaline hydrolysis of the substrate, so that it is difficult to assign a value to the action of the extract. Below 2.4 there was acid hydrolysis, but the effect on the gelatin was enough unlike that of the enzyme to make a clear differentiation. The speed of digestion at the various pH values is shown in Fig. 1. It will be noted that there is a peak at pH 3.6–4.0 and a much greater one at 8.0–8.49. The "speed of digestion" as shown in the figure is the reciprocal of the logarithm of the time for complete digestion by the most active lot of extract. As this is only comparative, no numerical scale is given.

Amylase

The method of Pickford and Dorris was used for this enzyme also, cornstarch being the substrate. The complete range of pH values was not tested, but an optimum was found at about 7.2, with a very rapid falling off of activity at 6.0 and a less rapid falling off at 8.0.

Lipase

The ethyl butyrate method previously used by me (1933) was adopted, but without the addition of water. Under the conditions of the experiment, the litmus turned pink only after a few days, but by the end of a week, the difference between the experimental and control tubes was extremely striking.

Alginase

Dr. Selman A. Waksman and his associates have become interested in the polymannuronic anhydride known as alginic acid. This (principally in the form of Ca and Mg salts) may make up 20–30 per cent of the air-dried weight of various marine algæ, such as *Fucus, Sargassum,*

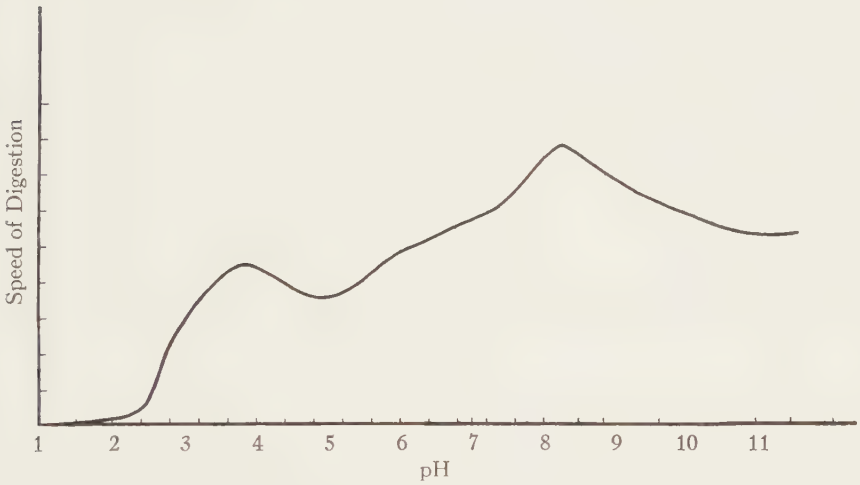

FIG. 1. Graph showing the action of the proteinase of *Calanus* on gelatin.

etc., and it unquestionably is a very important fraction of the total production of marine plants, corresponding to the stored carbohydrates of terrestrial plants. Of course these larger algæ are not ingested by *Calanus* (except perhaps in the form of fine detritus), but their composition may possibly give some indication of the makeup of the unicellular forms. In any case, it was considered desirable to attempt to discover an alginase in *Calanus*. Dr. Waksman kindly provided me a supply of alginic acid, and also a strain of alginic-acid-digesting bacteria, *Bacterium alginovorum* [Cf. Waksman, Carey, and Allen (1934)].

Agar plate methods were found quite unsuited for the purpose, because neither calcium alginate nor alginic acid remains unchanged by the buffers alone near the neutral point. In dry plates, moreover, the acid becomes practically invisible, and no specific stain is known to me for alginic acid, or its breakdown products.

A set-up was made, however, of 2 cc. of 3 per cent faintly alkaline sodium alginate solution, 1 cc. of buffer at pH 8.0, and 1 cc. of *Calanus* extract. A control with boiled extract was used. Two cc. of the liquid was allowed to drop from a pipette with a finely drawn-out tip, and the meniscus timed between marks with a stop-watch. There was not the slightest detectable change in viscosity during a period of 18 days.

The relative strengths of the enzymes investigated cannot be surely told, because the methods used are not comparable. However, a very dilute extract prepared in a preliminary experiment gave good starch digestion in 20–30 minutes at pH 7.0, whereas no digestion of gelatin occurred at any pH during 16 hours. From this it would certainly appear that the carbohydrate-splitting enzyme is much more vigorous than the proteinase—at least in the latter's action on gelatin.

Digestion of Whole Organisms

Suspensions of living *Nitzschia closterium* (Plymouth strain), *Dunaliella salina,* and *Bacterium alginovorum* were placed in drops of enzyme extract at pH 8.0 in depression culture dishes of soft glass, and the liquid covered by a layer of toluene (which, of course, killed the organisms). The culture dishes were kept at room temperature in Petri dishes containing water and toluene, and the toluene over the extract and in the Petri dishes was renewed from time to time. The controls were in the buffer without extract.

Some of the observations cited above show that *Calanus* guts often contain large numbers of diatoms. *Nitzschia* may be taken as a representative of this group of algæ (though not in itself an important constituent of *Calanus* food). In 36 hours the diatoms in the buffer alone appeared almost normal and stained well—practically like living organisms. In the enzyme, however, the tests appeared empty except for a minute refractive body in the center which took methylene blue extremely lightly—the rest of the diatoms remaining unstained.

The green flagellates after 36 hours were badly broken down both in the enzyme and the control, though those in the enzyme appeared slightly more disintegrated. But, after only 20–24 hours, there was a distinct difference: most of those in the control appearing approximately normal, whereas in the extract, although the chloroplast and other cell inclusions were not noticeably changed, they were crowded more or less into one end of the cell, leaving large clear areas in which no cytoplasm could be made out either without or with methylene blue staining.

The bacteria in the buffer control had settled in 36 hours to the bottom of the drop, and formed a slimy mass, much as they do when alive. In the enzyme the bacteria were perceptibly reduced in numbers (by

inspection, to about one-fourth of the control) and were perfectly separate and non-slimy. The individuals remaining, however, took methylene blue very much as the controls did.

Conclusions

Calanus finmarchicus contains enzymes which digest protein, starch, and fat. It has not been shown that these enzymes occur in the lumen of the gut, but that they do is certainly not improbable. Proteolytic digestion is performed by the extracted enzymes from pH 2.4 to above pH 11.0; there is a peak of activity at pH 3.6–4.0, and a still greater peak at pH 8.0–8.49.

Digestion of starch is performed by the extract from slightly below pH 6.0 to above pH 8.0, with an optimum at about 7.2. There is no digestion of alginic acid at pH 8.0, and no evidence was obtained that it is digested at any other pH.

The copepod extract will digest diatoms, bacteria, and at least a part of the cell contents of a green flagellate.

I wish to tender my thanks to the staff of the Woods Hole Oceanographic Institution for the grant of a summer fellowship, and for much invaluable advice and assistance in this work.

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Contribution No. 61

Canadian Atlantic Fauna

12. CHORDATA

12d. MARSIPOBRANCHII (LAMPREYS)
12e. ELASMOBRANCHII (SHARKS AND RAYS)
12f. HOLOCEPHALI (CHIMAEROIDS)

BY

HENRY B. BIGELOW AND W. C. SCHROEDER

WITH FIGURES

(Contribution No. 61 of the Woods Hole Oceanographic Institution)

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1934

MARSIPOBRANCHII, ELASMOBRANCHII ET HOLOCEPHALI

The numbers placed after the specific names refer to the numbered articles in the list of selected literature. Those in heavy type refer to articles containing descriptions or figures of the species.

Specimens of all, except the starred (*) species have been available.

SELECTED LITERATURE*

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KEY TO MAJOR DIVISIONS OF CANADIAN ATLANTIC FISHES

1. (2) Mouth soft, without articulated jaws; no paired fins.
   Class MARSIPOBRANCHII (p. 2)
2. (1) Mouth with well-developed jaws; pectoral fins present in all Canadian Atlantic species.
   Class PISCES (p. 4)
3. (6) Skull and skeleton cartilaginous; teeth not imbedded in the jaws.

*The nomenclature employed here follows Jordan and Everman (7) for the lampreys; Garman (4) for the sharks, skates and rays.

4. (5) Five or more pairs of large gill openings on each side of the neck.
Sub-class ELASMOBRANCHII (p. 4)
5. (4) Only one gill opening on each side of the neck. Sub-class HOLOCEPHALI (p. 38)
6. (3) Skull largely bony; teeth (if present) imbedded in the jaws.
Sub-class TELEOSTOMI (in later part)

MARSIPOBRANCHII. LAMPREYS AND HAGS

Mouth jawless situated at tip of snout, form eel-like, no paired fins. Skeleton cartilaginous, scarcely separated into skull and vertebral column. No ribs, shoulder, or pelvic girdles. Nostril single, median; skin naked.

KEY TO GENERA

1. (2) Only one fin on the back; snout with barbels, only one gill opening on each side. **MYXINE** (p. 2)
2. (1) Two separate dorsal fins; snout without barbels; seven gill openings on each side. **PETROMYZON** (p. 3)

MYXINIDAE. HAGFISHES

One gill opening on each side; snout with several barbels; dorsal fin-fold continuous.

Genus **MYXINE** Linnaeus
Characters as above.

M. glutinosa Linnaeus. Hagfish. **2**, 3, 5 (*Myxine limosa*), **7, 8.** (Fig. 1)

Body slender, eel-like, about 24 times as long as thick. Mouth with 2 barbels on each side; stellate in outline when closed; no lips; nostril surrounded by 4 barbels; tongue evertible, with two rows of 7-9 and 8-10 rasplike horny "teeth". Continuous fin-fold extends from posterior third of back, around tip of tail, and forward two-thirds the way along lower surface. A series of mucous sacs, with prominent pores, on either side of abdomen, extending from about one-thirteenth the way back from snout, to beyond cloaca. Pores 26-33 in front of branchial apertures; 57-66 between branchial aperture and cloaca (up to 70 recorded), and 11-13 behind cloaca, on 9 specimens from Grand Manan. In European specimens 24-34+54-64+10-14 are recorded. Both European and American specimens examined. Colour, grayish, to brownish, or reddish above, mottled or plain; whitish or pale gray below. Length up to 18 inches.

Depth 20 to 525 fathoms. Mud bottom. Greenland and Grand banks to North Carolina; most plentiful north and east of cape Cod; in Europe from White sea to English channel.

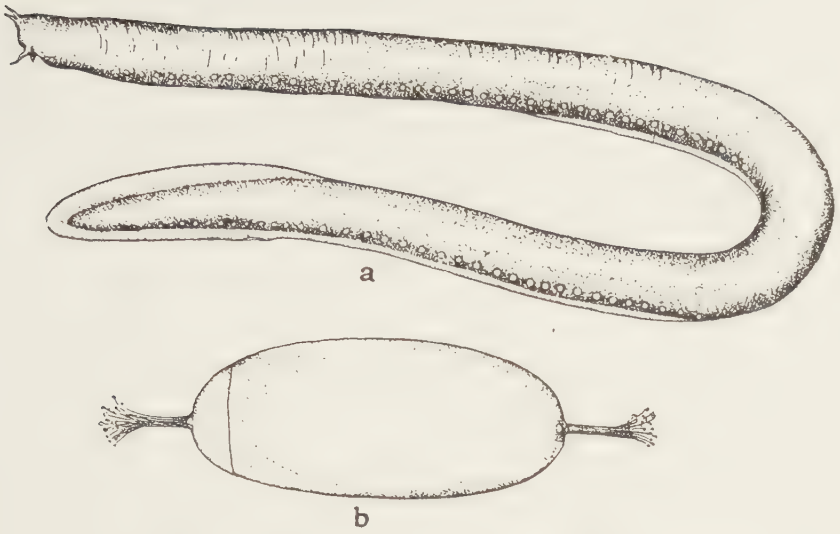

Fig. 1. Hagfish (*Myxine glutinosa*). a, Adult. b, Egg. After Bigelow and Welsh.

PETROMYZONTIDAE. LAMPREYS

Gill openings 7 on each side; snout without barbels; dorsal portion of fin-fold deeply notched or divided into two dorsal fins; rear part continuous around the tail.

Genus **PETROMYZON** Linnaeus

Buccal disk large, with numerous teeth arranged in concentric series. Dorsal fin-fold divided into two separate fins.

P. marinus Linnaeus. Sea lamprey. **2**, 3, 5, **7**, **8**. (Fig. 2)

Body eel-like. Skin naked. Mouth a longitudinal slit when closed, an elliptical disk when open; anterior lingual lamella sharply curved, but without differentiated median cusp. Gill clefts 7 on each side. Horny teeth numerous, in 11 or 12 concentric rows in radiating series; innermost teeth the largest. Dorsal fin-fold well separated, the first originating near middle of body, the second continuous around tail with ventral fold. No paired fins. Colour, mottled

Fig. 2. Sea lamprey (*Petromyzon marinus*). After Jordan and Evermann

brown above, sometimes greenish, reddish, or plain blue; below whitish, gray, or a paler shade of ground colour of back. Length usually less than $2\frac{1}{2}$, rarely up to 3 feet.

Anadromous, ascending freshwater streams in spring to spawn. West Greenland and southern Labrador to Florida; Iceland; Norway and Faroes south to Mediterranean and west Africa.

PISCES. TRUE FISHES

ELASMOBRANCHII. SHARKS AND RAYS

Five or more pairs of gill openings; skin leathery, with denticles (placoid scales). Fins supported by cartilaginous rods, and horny fibres, and covered by the leathery skin. Skeleton chiefly cartilaginous; skull far simpler than in bony fishes; jaws and teeth highly developed, the latter imbedded in the gums, with successive rows becoming functional. Gills attached throughout their lengths to the partitions between the gill clefts.

Inner margins of ventral fins modified into rod-like, semi-tubular copulatory organs, in males.

KEY TO GENERA (ADULTS)

1.	(32)	General form cylindrical.	(*Sharks*)	
2.	(3)	Head hammer- or shovel-shaped.	**CESTRACION**	(p. 9)
3.	(2)	Head of ordinary shape; rounded or pointed snout.		
4.	(5)	One dorsal fin; 6 gill clefts.	**CHLAMYDOSELACHUS**	(p. 5)
5.	(4)	Two dorsal fins; 5 gill clefts.		
6.	(15)	No anal fin.		
7.	(8)	First dorsal far back, over ventrals.	**ECHINORHINUS**	(p. 20)
8.	(7)	First dorsal well in front of ventrals.		
9.	(14)	First and second dorsals each preceded by a spine, long or very short.		
10.	(13)	Dorsal spines long.		
11.	(12)	Rear margin of upper lobe of tail notched near tip.	**CENTROSCYLLIUM**	(p. 18)
12.	(11)	Rear margin of upper lobe of tail not notched.	**SQUALUS**	(p. 17)
13.	(10)	Dorsal spines very short.	**CENTROSCYMNUS**	(p. 17)
14.	(9)	No dorsal spines.	**SOMNIOSUS**	(p. 19)
15.	(6)	Anal fin present.		
16.	(17)	Gill clefts very long, nearly meeting below; gills with horny rakers; teeth tiny.	**CETORHINUS**	(p. 16)
17.	(16)	Gill clefts confined to sides of neck; gills without rakers; teeth large.		
18.	(21)	Caudal peduncle with a strong longitudinal keel on either side; tail lunate with lower lobe at least two-thirds as long as upper.		
19.	(20)	Teeth broad, triangular, with serrate edges; pectoral $1\frac{1}{2}$ to 2 times as long as first dorsal is high.	**CARCHARODON**	(p. 15)
20.	(19)	Teeth slender, lanceolate, pectoral only slightly longer than first dorsal is high.	**ISURUS**	(p. 13)
21.	(18)	No longitudinal keels on caudal peduncle; upper lobe of tail much longer than lower lobe.		

22. (23) Upper caudal lobe nearly as long as head and body combined. **ALOPIAS** (p. 11)
23. (22) Upper caudal lobe less than half as long as head and body combined.
24. (25) Teeth small, blunt, and arranged in a pavement. **MUSTELUS** (p. 6)
25. (24) Teeth erect, pointed, and only one or two rows functional at a time.
26. (27) Second dorsal nearly as high as first. **CARCHARIAS** (p. 12)
27. (26) Second dorsal less than half as high as first.
28. (29) Origin of first dorsal close behind axilla of pectorals; outer margins of teeth deeply notched. **GALEOCERDO** (p. 8)
29. (28) Origin of first dorsal considerably behind axilla of pectorals; outer margins of teeth not deeply notched.
30. (31) First dorsal originates nearer to ventrals than to pectorals; snout long, pointed. **PRIONACE** (p. 7)
31. (30) First dorsal originates nearer to pectorals than to ventrals; snout short, rounded. **CARCHARINUS** (p. 7)
32. (1) General form flat and disk-like. (*Skates and Rays*)
33. (36) No long dorsal spines on tail; extremity of tail comparatively stout.
34. (35) Caudal fin very small; skin more or less prickly. **RAJA** (p. 21)
35. (34) Caudal fin large, triangular, skin perfectly smooth. **NARCACION** (p. 35)
36. (33) Tail with one or more long dorsal spines. **DASYBATUS** (p. 36)

CHLAMYDOSELACHIDAE. EEL SHARKS

Six gill clefts; 1 dorsal fin without spine; no nictitating membrane; nostrils on top of snout.

Genus **CHLAMYDOSELACHUS** Garman

Characters as above.

C. anguineus Garman. Eel shark. **2**, 3, **4**, **7**. (Fig. 3)

Body eel-like; mouth terminal. Six pairs of gill clefts. Teeth similar in the two jaws, with a broad base and 3 curved, slender cusps; $\frac{28}{26}$ to $\frac{32}{32}$ rows; one median tooth. Dorsal fin far back, low, somewhat smaller than and above anal. Caudal pointed. Colour, uniform brown. Length 2 to 5 feet.

Japan; New South Wales; Madeira; Norway; and doubtful record from Pemaquid point, Maine.

Fig. 3. Eel shark (*Chlamydoselachus anguineus*). After Goode and Bean.

MUSTELIDAE.[1] SMOOTH DOGFISHES

Five gill clefts; 2 dorsal fins without spines; anal fin present; axis of tail little raised; nictitating membrane present; teeth in bands, or in pavement, several series functioning at once.

Genus **MUSTELUS**[1] Linck

Teeth blunt, in pavement. No nasoral grooves.

M. canis (Mitchill). Smooth dogfish. **2** and **4** (*Galeorhinus laevis*), **7**. (Fig. 4)

Trunk slender; snout flattened, blunt at tip; mouth crescentic; teeth small, low, blunt, rhomboid to oval, their upper surface convex, some with outer edges more or less deeply notched, arranged like a pavement, $\frac{74}{80}$ rows in specimen examined. First dorsal triangular, its rear margin concave, originating close behind axilla of pectorals. Second dorsal similar to first, about two-thirds as high, situated far back, but originating in front of anal. Lower caudal lobe only about one-fourth as long as upper, rear margin of upper deeply notched. Pectorals about as large as first dorsal. Colour, plain gray or olive to brown sometimes with pale spots above; white yellowish, or pale gray below. Length of adults usually 2 or 3 feet; maximum about 5 feet.

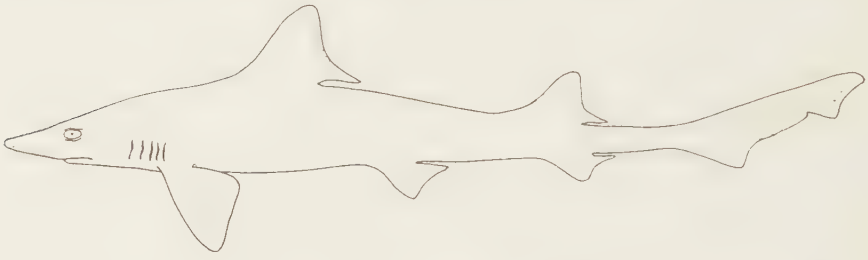

Fig. 4. Smooth dogfish (*Mustelus canis*). After Garman.

Teeth.

Shoal water. Bay of Fundy to Cuba, not common north of cape Cod; Atlantic coasts of southern Europe; Mediterranean; South Africa from the cape to Natal.

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[1]Opinion 93, International Commission on Zoological Nomenclature (Science, N. S., vol. 65, p. 300) places *Mustelus* in the official list. Consequently *Mustelidae* replaces *Galeorhinidae* as the family name.

CARCHARINIDAE. REQUIEM SHARKS

Teeth compressed, sub-triangular, sharp-edged, pointed, with a single large cusp, one series functional at a time; otherwise as in Mustelidae (p. 6).

Genus **CARCHARINUS**

First dorsal (western Atlantic species) originates nearer to axilla of the pectorals than to ventrals. No spiracle. Some or all of the teeth serrate; their cusps erect, or only slightly oblique.

C. obscurus* Lesueur. Dusky shark. **2**, **4**, **7**. (Fig. 5)

Trunk heaviest at or behind the pectorals; snout blunt, broader than long, rounded at tip; short grooves at corners of mouth. Teeth erect, separate, with serrate margins, in about $\frac{29}{30}$ rows; upper triangular, inner margin convex, outer notched; lower teeth with narrow lanceolate cusps and broad bases. Height

Fig. 5. Dusky shark (*Carcharinus obscurus*). After LeSueur, slightly emended.

of first dorsal nearly equal to distance from eye to first gill cleft, origin behind inner angle of pectoral. Second dorsal above anal, noticeably smaller than latter. Upper caudal lobe much the larger; pectorals relatively long and narrow. Colour, gray brown above; whitish below. Length to about 14 feet, usually 6 to 9 feet.

Teeth, after LeSueur.

Coastal waters, north casually to cape Elizabeth, Maine, and northeastern part of Georges bank; southern range doubtful because of confusion with other species. Madeira; Canary islands; South Africa.

Genus **PRIONACE** Linnaeus

First dorsal nearer to ventrals than to pectorals; teeth serrate, unlike in the two jaws; no spiracles.

P. glauca (Linnaeus). Great blue shark. **1, 2, 4** (*Galeus glaucus*); 5, **7** (*P. glauca*); **8** (*Carcharias glaucus*). (Fig. 6)

Body fusiform, rather slender. Snout conical, pointed. Mouth crescentic, teeth usually $\frac{28}{28}$ to $\frac{31}{31}$, exceptionally as few as 23 rows in lower jaw; upper teeth triangular, serrate, curved outward, inner margins convex, outer margins concave; lower teeth narrower, with nearly straight cusps and weaker marginal serrations.

Teeth.

Fig. 6. Great blue shark (*Prionace glauca*). Based on photographs of specimens taken on Georges bank.

Teeth more strongly serrated in small than large fish. First dorsal notably small, height equal to about snout to eye, origin nearer ventrals than axilla of pectorals. Second dorsal less than half as high as first, over anal. Lower caudal lobe about half as long as upper, each measured from the corresponding caudal pit. Pectorals, long, scimitar-shaped, length equal to about snout to third gill cleft. Colour, strong blue[2] above, shading to paler on sides; white below, tips of pectorals usually dusky; anal sometimes partly dusky. Maximum length in northern seas usually 10 to 12 feet.

Pelagic in all warm oceans. North to Nantucket shoals and Nova Scotia, and occasionally to Grand banks.

Genus **GALEOCERDO**

First dorsal much closer to pectorals than to ventrals. Teeth serrate, alike in the two jaws. Small spiracles present.

---

[2]"Sailor Blue," plate XXI, Color Standards and Color Nomenclature, Robert Ridgway, 1912, Washington.

G. arcticus (Faber). Tiger shark. **2, 4, 7** (*G. tigrinus*). (Fig. 7)

Body heaviest forward of first dorsal, tapering toward tail; snout short, broad, rounded; mouth nearly two-thirds breadth of snout; labial folds much longer on

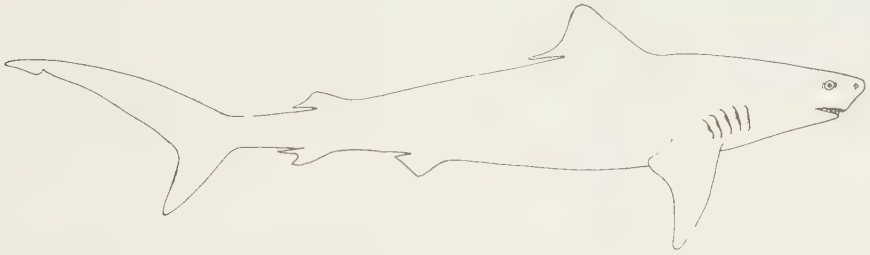


Fig. 7. Tiger shark (*Galeocerdo arcticus*). From a specimen 4½ feet long in the Museum of Comparative Zoölogy.

upper jaw than on lower. Teeth large, sharp-edged, alike in the two jaws, in $\frac{21}{21}$ to $\frac{25}{25}$ rows; points directed toward the corners of the mouth, inner edges convex, outer edges concave and deeply notched; both edges with coarse serrations near the base, but nearly smooth near tip. First dorsal triangular, slightly smaller than pectorals; originating very slightly behind axilla of pectoral in specimen examined (its position variously described). Second dorsal and anal very small, about equal, the former standing before the latter. Pectorals as long as distance from eye to third gill cleft; upper caudal lobe notched, about twice length of lower, or one-fourth length of fish. Caudal peduncle with low keels. Colour, light brown, more or less spotted and barred with darker brown, these markings fading with growth. Length frequently 12 to 15 feet; exceptionally to 30 feet.

Teeth.

Cosmopolitan in warm parts of all oceans; chiefly coastal; not uncommon north to Woods Hole; occasional at Provincetown, cape Cod.

CESTRACIONTIDAE. HAMMERHEAD SHARKS

Characters as in Carcharinidae, except frontal region of skull expanded laterally, in characteristic "heart" or "hammer" outline, with the eyes at the tips.

Genus **CESTRACION**

Characters as above.

Key to species

Head very broad, its greatest width about 3 in total length.	**zygæna**
Head less broadly expanded, its width about 5 in total length.	**tiburo**

C. zygæna (Linnaeus). Hammerhead. **2, 4, 7** (*Sphyrna zygæna*). (Fig. 8)

Trunk slender. Head hammer-shaped with the eyes at ends of lateral processes. Teeth in $\frac{29}{28}$ to $\frac{36}{34}$ rows; similar in the two jaws; usually oblique, sometimes nearly erect, margins nearly straight, outer notched, more or less serrate (fig. 8); lower narrower than upper. First dorsal higher than length of its base, origin well behind axilla of pectoral; second dorsal hardly one-fifth height of first, above anal, which is similar. Upper caudal lobe (measured from its pit) notably long, equal to about 30 per cent of total length of fish; deeply notched near its tip; lower lobe less than half length of upper; pectorals broadly triangular, smaller than first dorsal. Colour, gray to ashy brown above; paler brown to dirty white below. Maximum length 17 feet.

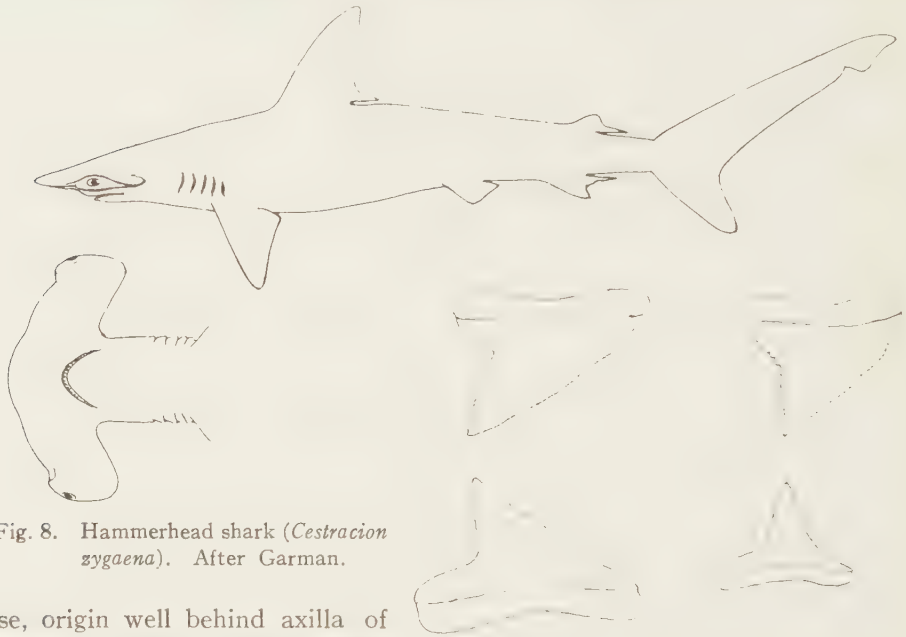


Fig. 8. Hammerhead shark (*Cestracion zygaena*). After Garman.

Upper and lower teeth of two specimens, showing individual variation.

Cosmopolitan in warm coastal seas; north to offing of southern Nova Scotia and England.

C. tiburo (Linnaeus). Bonnet shark. **4, 7** (*Sphyrna tiburo*). (Fig. 9)

Body moderately slender, compressed; lateral expansions of head shorter and relatively much broader than in *C. zygæna*. Mouth crescentic. Teeth in $\frac{26}{25}$ to $\frac{32}{31}$ rows, with broad bases, and low cusps, upper more oblique than lower.

First dorsal origin behind axilla of pectoral, height greater than length of base; second dorsal and anal relatively larger than in *C. zygæna*, nearly one-third as high as first dorsal. Caudal long, upper caudal lobe deeply notched near tip, lower lobe less than half length of upper. Ventrals larger than in *C. zygæna*;

Fig. 9. Bonnet shark (*Cestracion tiburo*). After Garman.

length of pectorals about equal to height of first dorsal, broader than in *C. zygæna*. Colour, grayish above, sometimes with a few small dark spots on sides. Pale below. Length seldom more than 5 feet.

Widespread in warm, coastal seas. Casual to Massachusetts bay.

ALOPIIDAE. THRESHER SHARKS

Characters as in Carcharinidae (p. 7), except tail enormously elongate, and no nictitating membrane.

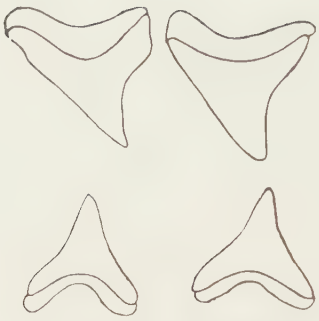
Genus **ALOPIAS**

Characters those of the family.

A. vulpinus Bonnaterre). Thresher. 2 and 4 (*Vulpecula marina*), 5, 7 and 8 (*A. vulpes*). (Fig. 10)

Body stoutest opposite pectorals; snout broadly rounded, notably short;

Fig. 10. Thresher (*Alopias vulpinus*). After Garman.



Teeth of *A. vulpinus*.

teeth in $\frac{42}{37}$ to $\frac{50}{50}$ rows; alike in the two jaws, small, triangular, with smooth edges. First dorsal relatively low, origin well behind axilla of pectoral. Second dorsal very small, similar to and in front of anal. Caudal enormously long, scythe-shaped, the peduncle without keels or pits. Ventrals much larger than anal, about as long as lower caudal lobe; pectorals sickle-shaped, length about twice height of first dorsal. Colour, dark leaden brown to nearly black above; white below; lower sides of pectorals leaden. Maximum length 20 feet or more.

All warm seas, north to Nova Scotia, and perhaps gulf of St. Lawrence.

CARCHARIIDAE. SAND SHARKS

Characters as in Carcharinidae (p. 7), except no nictitating membrane.

Genus **CARCHARIAS**

Snout short; upper caudal pit well developed; lower caudal lobe well developed.

C. taurus Rafinesque. Sand shark. **2, 4, 7** (*C. littoralis*). (Fig. 11)

Body stout; snout short, flattened above, blunt at tip. Teeth in $\frac{40}{36}$ to $\frac{46}{40}$ rows; alike in the two jaws, long, narrow, pointed, smooth-edged, most of them with a denticle at either side near base. First dorsal, originating well behind inner angle of pectoral, relatively small, height about equal to snout to eye, its base equal to height. Second dorsal and anal, similar to first dorsal, and nearly as large. Upper caudal lobe deeply notched, about 30 per cent total length of fish; lower lobe about one-third length of upper. Pectorals triangular, slightly larger than first dorsal. Colour, gray, darker above than below, indistinctly spotted or mottled with darker; fins sometimes edged with black. Length of adults usually 4 to 6 feet, rarely 8 or 9 feet.

Teeth of *C. taurus*.

Shoal coastal waters, North Carolina to gulf of Maine, casual to bay of Fundy; Mediterranean and South Africa.

Fig. 11. Sand shark (*Carcharias taurus*). After Garman.

ISURIDAE. MACKEREL SHARKS

Anal fin present; no dorsal spines. Vertebral axis of tail turns abruptly upward from horizontal; lower caudal lobe only slightly shorter than upper. A strong mid-lateral keel on either side of caudal peduncle; deep upper and lower caudal pits.

Genus **ISURUS**

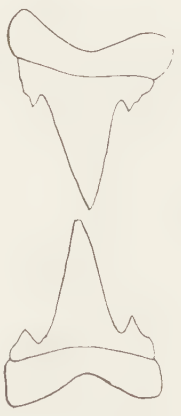
Gills without horny strainers. Teeth large, narrow, pointed, their margin not serrate.

Key to species

First dorsal origin well behind axilla of pectoral. Teeth without denticles. **tigris**
First dorsal origin over axilla of pectoral. Teeth with lateral denticles in adults. **nasus**

I. nasus (Bonnaterre). Common mackerel shark. **1, 2** (*I. punctatus*+*I. nasus*), 3 (*I. cornubicus*), **4** (*I. punctatus*+*I. nasus*), 5 and 7 (*Lamna cornubica*), **8** (*I. cornubicus*). (Fig. 12)

Trunk very stout, thickest opposite pectorals; snout conical, pointed; teeth in $\frac{24}{20}$ to $\frac{32}{28}$ rows, alike in the two jaws, with narrow pointed cusps, and broad bases, having small, sharp denticle on each side near base in adults; these denticles lacking in some or all teeth in young. Third tooth from centre in upper jaw very small. First dorsal arises directly over axilla of pectoral, triangular, about as high as long, height about equal to eye to second gill cleft. Second dorsal very small; origin over origin of anal. Anal similar to second dorsal. Tail lunate; upper caudal lobe (measured from its pit) nearly one-third distance from snout to caudal base, its rear margin notched near tip; lower caudal lobe about 70 per cent as long as upper. Pectorals longer than first dorsal is high; falcate-triangular. Colour, dark bluish gray above; white below; pectorals dusky, on outer half or one-third. Anal white or slightly dusky. Usual maximum length about 12 feet.



Teeth of *I. nasus*.

Fig. 12. Common mackerel shark (*Isurus nasus*). After Garman.

Pelagic in north Atlantic, Mediterranean and north Pacific; regularly north to Maine, occasional to the gulf of St. Lawrence and to southeastern Newfoundland.

I. tigris (Atwood). Sharp-nosed mackerel shark. **1, 2, 4, 7** (*I. dekayi*). (Fig. 13)

Trunk fusiform, snout conical, more pointed than in *I. nasus*. Teeth in about $\frac{26}{26}$ rows; alike in the two jaws, with narrow, pointed cusps, broad-based,

Fig. 13. Sharp-nosed mackerel shark (*Isurus tigris*) from a painting by J. Henry Blake, of a Massachusetts bay specimen, 8 ft. 4 in. long.

First four teeth from centre of upper and lower jaw. After Murphy.

without lateral denticles. First two teeth from the median line of the mouth longest, and more flexuous than the others. Third upper tooth smaller than the fourth. First dorsal, arising over rear angle of pectoral, relatively shorter than in *I. nasus*; origin of second dorsal anterior to anal origin. Lower lobe of caudal relatively longer than in *I. nasus*, about 75 per cent as long as upper lobe, each measured

from caudal pit. Colour, dark clear blue to ashy brown above; white below, with demarcation sharply defined. Pectoral without dusky blotch at the tip. Maximum length about 10 feet.

Pelagic in western Atlantic; West Indies to Massachusetts bay; perhaps identical with *I. oxyrhynchus* of Mediterranean and eastern Atlantic.

Genus **CARCHARODON**

Gills without horny strainers; teeth large, triangular, with serrate margins.

C. carcharias (Linnaeus). White shark. **2, 4,** 5, **7.** (Fig. 14)

Trunk fusiform; snout conical, relatively shorter than in *Isurus*; distance

Fig. 14. White shark (*Carcharodon carcharias*). After Garman.

Teeth.

from its tip to eyes same as to mouth; teeth in about $\frac{26}{24}$ rows, triangular, serrate, lower narrower than upper, with edges more concave. First dorsal origin slightly behind axilla of pectoral; base slightly longer than height; height about equal to distance from corner of mouth to first gill cleft. Second dorsal very small, entirely in front of anal. Pectorals large, falciform, length equal to distance from snout to first gill cleft. Caudal lunate; lower lobe 73 to 92 per cent as long as upper, measured from pit; upper lobe notched. Caudal peduncle with deep pit above, a shallow below; with well developed longitudinal keels. Colour, slaty, leaden or blue-gray above; white below; a black spot in axilla of pectoral, sometimes extending out on fin, and on trunk. Maximum length to 40 feet; northern specimens usually small.

Cosmopolitan in warm seas; nowhere abundant; occasional in Massachusetts bay, bay of Fundy, and probably southeastern Nova Scotia.

Genus **CETORHINUS**

Gills with horny strainers; gill clefts very long; teeth small, conical.

C. maximus (Gunner). Basking shark. **2**, 3, **4**, 5, **7**, **8**. (Fig. 15)

Trunk stout; snout, in adult, blunt conical, flattened above (14 inches in front of mouth in 26 ft. specimen): in young produced in front of mouth into a sub-cylindrical proboscis (**8**, fig. 331) pointed at tip;

Fig. 15. Basking shark (*Cetorhinus maximus*), from a specimen about 26 feet long, in Boston Society of Natural History.

(about 9 inches long in 8-ft. Japanese specimen examined). Teeth minute, numerous, $\frac{200}{200}$ rows recorded, conical, points directed inwards; several rows functional at once. Skin of throat loose, widely distensible. First dorsal originates well behind posterior angle of pectoral, height equal to about one-twelfth total length; triangular. Second dorsal and anal very small, former originating in front of latter, similar, their height equaling about one-fourth length of first dorsal. Caudal in adult of Isurid form, margin notched; lower lobe about two-thirds length of upper; lower lobe relatively shorter in young (**8**, fig. 331a), about half upper in 8 ft. specimen. Caudal peduncle with shallow upper and lower caudal pits; its sides described as with strong longitudinal keels. Pectorals much longer than first dorsal is high. Colour, grayish brown to slaty above; under parts nearly the same or paler, uniform or shading into white, sometimes with white patch under snout. Maximum size at least 40 feet, often 25 to 35 feet.

North Atlantic northward to gulf of Maine, Newfoundland, Iceland, and northern Norway; Mediterranean; north and south Pacific; Australian region; South Africa.

SQUALIDAE. SPINY DOGFISHES

No anal fin. Two dorsal fins, with sharp spines.

Genus **SQUALUS**

Rear margin of the upper caudal lobe not notched; teeth alike in the two jaws; dorsal spines long.

S. acanthias. Linnaeus. Spiny dogfish. **2**, 3, **4**, 5, 6, **7**, **8**. (Fig. 16)

Trunk slender; snout flattened above, rounded at tip; long diameter of eye equals about half eye to snout; teeth in about $\frac{26}{26}$ rows; similar in the two jaws;

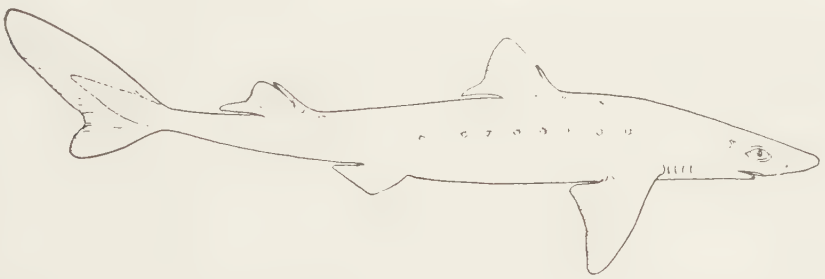


Fig. 16. Spiny dogfish (*Squalus acanthias*). After Garman.

Teeth.

outer margins notched, points turned towards corners of mouth. First dorsal originates behind rear angle of pectoral; triangular, its rear margin only slightly concave; with a smooth spine less than half length of fin. Second dorsal about two-thirds as high as first, with a smooth spine nearly as high as the fin. Lower caudal lobe about 40 to 45 per cent as long as upper. A low, longitudinal fold of skin on each side of caudal peduncle; no caudal pits. No anal. Ventrals well in front of second dorsal. Pectorals large, triangular, broader than long. Colour, slate coloured, rarely brown above; young with a row of small white spots from pectoral to anal; other spots in front of and behind first dorsal and in front of second; the spots fading with age. Gray to white below. Length of mature males up to about 3 feet; females to about $3\frac{1}{2}$ feet.

Both sides of north Atlantic; Mediterranean; South Africa; west Greenland and strait of Belle Isle to Cuba in western Atlantic; most plentiful North Carolina to gulf of St. Lawrence.

Genus **CENTROSCYMNUS**

Caudal notched; teeth unlike in the two jaws; each tooth with only one cusp; dorsals each with a very small spine; eyes large.

C. coelolepis Bocage and Capello. Portuguese shark. **2, 4,** 5, **7.** (Fig. 17)

Snout short, rounded; eye large, diameter equal to about 60 per cent distance from snout to eye; teeth in about $\frac{70}{42}$ rows, upper narrow, lanceolate; lower broad, flat, rectangular, outer margins notched, forming a cutting edge. First dorsal far back, about midway between pectorals and ventrals, notably small, height about equals snout to eye. Second dorsal slightly larger than first (smaller in most other sharks). Upper caudal lobe roughly rectangular, notched, lower lobe about half length of upper. No anal fin. Pectorals small, rear margin nearly straight; angles scarcely rounded. Colour, deep chestnut or blackish brown, above and below. Length, 3 to 4 feet.

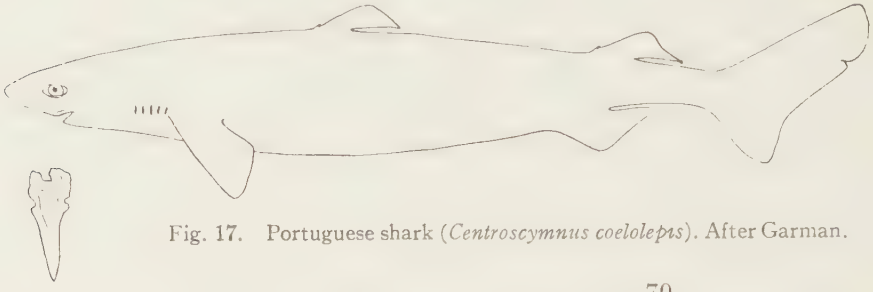


Fig. 17. Portuguese shark (*Centroscymnus coelolepis*). After Garman.

Teeth.

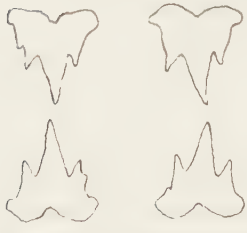
Usually deeper than 150 fathoms. Grand bank, La Have, Georges; Iceland, the Faroes, Portugal, Madeira, Mediterranean, Japan.

Genus **CENTROSCYLLIUM**

Dorsal spines long, grooved longitudinally on each side; teeth alike in the two jaws; each with several cusps; caudal notched in Atlantic species.

C. fabricii (Reinhardt). Black dogfish. **2,** 3, **4,** 5, 6, **7.** (Fig. 18)

Trunk slender; snout short, bluntly rounded. Eye notably large, long diameter nearly equal to snout to eye; teeth in about $\frac{70}{70}$ rows, alike in the two jaws; each with 3 sharp triangular cusps, the central one longest. First dorsal small, its upper edge rounded; originating well behind rear angle of pectoral. Second dorsal larger than first, roughly triangular. Dorsal spines deeply grooved. No anal fin. Lower caudal lobe but weakly marked off from upper; about half as long. Rear axil of ventral under origin of second dorsal. Pectorals small, rounded. Colour, uniform black or dark brown, above and below. Length $2\frac{1}{2}$ to $3\frac{1}{2}$ feet.



Teeth of *C. fabricii*, after Garman.

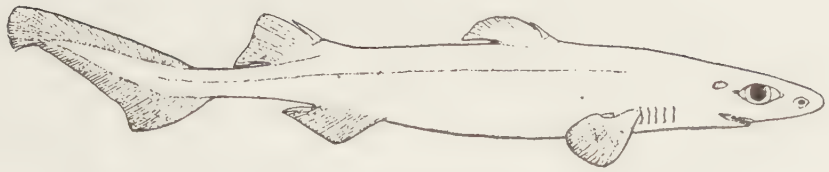


Fig. 18. Black dogfish (*Centroscyllium fabricii*). After Garman.

Usually deeper than 150 fathoms. West Greenland; slopes of Grand bank to Georges bank; gulf of Mexico; Faroes, Hebrides, Iceland; perhaps Falkland islands, Japan.

SCYMNORHINIDAE. NURSE SHARKS

No anal fin; two dorsal fins lacking spines in Atlantic genera; neither caudal pits nor lateral keels; teeth unlike in the two jaws; snout short.

Genus **SOMNIOSUS**

Second dorsal about as large as first: lower teeth forming a continuous cutting edge, facing the upper jaw; eyes notably small.

S. microcephalus (Bloch and Schneider). Greenland shark. 2, 3 (*Acanthorhinus carcharias*), **4**, 5, 6, **7**, **8** (*Acanthorhinus carcharias*). (Fig. 19)

Trunk moderately stout, head strongly arched above; snout short, flattened above, rounded at tip; diameter of eye less than one-third snout to eye; teeth in about $\frac{41}{48}$ rows, upper narrow, triangular to lancet-shaped, erect; lower quadrate,

Fig. 19. Greenland shark (*Somniosus microcephalus*). After Garman.

Teeth.

their tips sharply bent toward the corners of mouth, outer margins deeply notched, inner nearly straight. Height of first dorsal equal to about half distance from mouth to snout; origin midway between snout and base of caudal; posterior angle projecting rearward for a distance greater than height of the fin. Second dorsal similar to first, slightly smaller. Distance from axilla to origin of first dorsal equals 31 to 40 per cent of distance from axilla to ventrals.

Caudal axis only slightly raised; upper caudal lobe notched; the lower about 70 per cent as long. No caudal pits, nor keels. No anal fin. Pectorals very small, with rounded angles. Colour, gray, brownish or black, nearly as dark below as above, sometimes purplish; back and sides with many indistinct dark transverse bands; some specimens with white spots. Length of adults averages 8 to 12 feet; rarely to 18 feet.

Chiefly deeper than 20 to 30 fathoms, on muddy bottom. Arctic and sub-Arctic; south to cape Cod; to North sea and English channel; and to Oregon.

ECHINORHINIDAE. BRAMBLE SHARKS

No anal fin. Two dorsals without spines; neither caudal pits nor lateral keels; teeth similar in the two jaws, forming cutting edges.

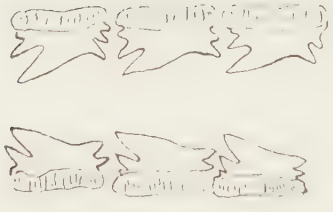
Genus **ECHINORHINUS**

Characters of the family.

E. brucus* (Bonnaterre). Bramble shark. **2, 4, 7** (*E. spinosus*). (Fig. 20)

Trunk moderately slender; snout short with rounded tip. Teeth alike in the two jaws; diagnostic in outline, the pointed cusps directed towards corners

Fig. 20. Bramble shark (*Echinorhinus brucus*). From Goode and Bean, after Day.



Teeth, after Moreau.

of mouth, each margin with one or two deep notches near its base. All fins small, as in *Somniosus*. First dorsal far back over ventrals. Second dorsal close behind first. Caudal axis sharply bent. No anal. Skin notably spinous. Colour, dark brown above, with or without darker blotches; paler brown to white below. Maximum recorded length, 9 feet.

Chiefly deeper than 100 fathoms. Great Britain to north Africa; Mediterranean; cape of Good Hope; New Zealand; Australia; Japan; accidental at Provincetown, cape Cod.

RAJIDAE. SKATES

General form flat, disk-like; tail moderately stout, without serrated dorsal spines; caudal fin present but very small; skin of the disk or tail with at least a few small spines, and often thorny.

Genus **RAJA**

Snout supported by a distinct cartilage; dorsal fins near end of tail; margins of pectorals not produced into sharp points; disk rhombic. Males with several bands of erectile hooks near the outer corners of the pectorals.

Key to species

1.	(2)	Lower surface with minute rounded tubercles.	**granulata**
2.	(1)	Lower surface smooth.	
3.	(6)	No large thorns along mid-dorsal zone of disk between eyes and ventral fins.	
4.	(5)	Mid line of disk densely provided with small spines.	**spinicauda**
5.	(4)	Mid line of disk smooth.	**stabuliforis**
6.	(3)	One or more rows of large thorns along mid-dorsal zone of disk behind eyes.	
7.	(8)	Posterior third of tail without thorns.	**senta**
8.	(7)	Thorns on tail extending at least to dorsal fin.	
9.	(18)	One row of large thorns on tail.	
10.	(11)	Mid row of thorns between eyes and first dorsal numbering about 47 to 50.	**lintea**
11.	(10)	Mid row of thorns less than about 45.	
12.	(13)	Lower surface of disk with irregular dark blotches.	**hypoboreas**
13.	(12)	Lower surface of disk white or grayish without dark blotches.	
14.	(17)	Mid row of thorns not exceeding 20.	
15.	(16)	Upper surface smooth between the thorns.	juvenile **scabrata**
16.	(15)	Upper surface prickly between the thorns.	**radiata**
17.	(14)	Mid row of thorns numbering about 34 to 37.	juvenile **fyllae**
18.	(9)	Two or more rows of thorns on tail.	
19.	(20)	Mid row of thorns on tail much larger than any others.	**scabrata**
20.	(19)	Mid row of thorns on tail not much larger than the others.	
21.	(22)	Upper surface of disk with many narrow irregular dark bars, as well as spots.	**eglanteria**
22.	(21)	Upper surface of disk either plain or spotted, but not barred.	
23.	(24)	Teeth in more than 65 rows in each jaw.	**diaphanes**
24.	(23)	Teeth in less than 65 rows.	
25.	(26)	Teeth in 38 to 60 rows; upper surface of disk with many small round dark spots; with or without a large spot on each pectoral.	**erinacea**
26.	(25)	Teeth in 30 to 36 rows; upper surface of disk plain; with or without a large spot on each pectoral.	**fyllae**

R. erinacea. Mitchill. Little skate. **2, 4,** 5, **7.** (Fig. 21)

Anterior outline obtuse. In specimens, 17 to 21 inches long, distance from snout to centre of eye equals 37 to 40 per cent the breadth of disk abreast the eyes; length of disk, from snout to rear angle of pectoral, equals 82 to 86 per cent of breadth of disk; and 48 to 51 per cent of total length. Tail about half total length. Teeth in $\frac{46}{48}$ to $\frac{50}{52}$ rows in 3 adult specimens examined: 38 to 60 rows reported.

Large thorns on back and sides of tail, shoulders, head and snout, in several irregular rows along the dorsal ridge, and scattered generally over upper surface of disk, but (except rarely) no definite mid-dorsal row much larger than the others. Colour, grayish to dark brown above, often clouded with lighter and darker, usually with many small round darker spots; sometimes with whitish eye-spots near rear angles of pectorals; white or pale grayish below. Maximum length about 24 inches.

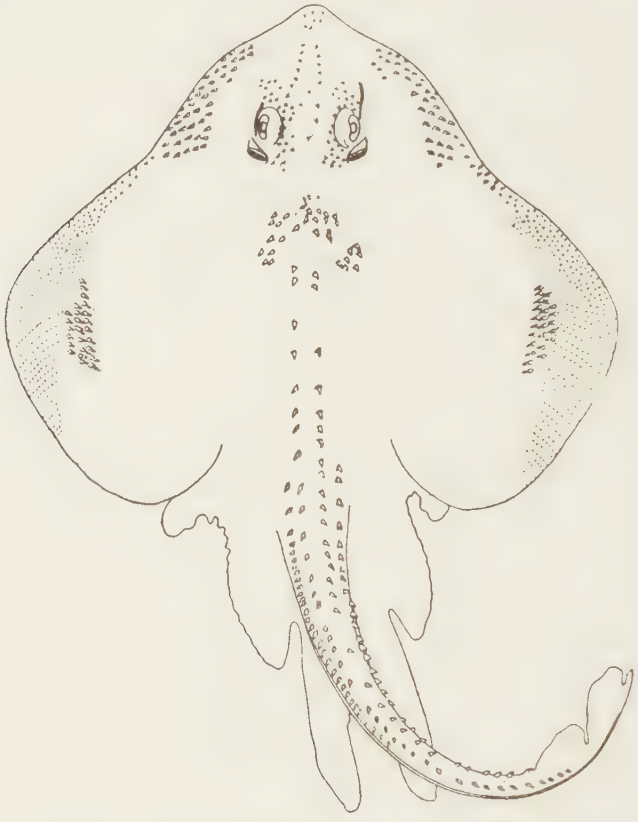


Fig. 21. Little skate (*Raja erinacea*). After Garman.

Tide line down to about 50 fathoms; commonest in shoal water. Continental shelf of eastern North America, Scotian banks and southern side of gulf of St. Lawrence to Chesapeake bay; mostly shoal.

R. diaphanes Mitchill. Spotted skate. **2, 4,** 5 and **7** (*R. ocellata*). (Fig. 22)

Anterior outline obtuse; snout short. In specimens $18\frac{1}{2}$ to 36 inches long distance from snout to centre of eye equals 36 to 38 per cent of breadth of disk abreast the eyes; length of disk from snout to rear angle of pectoral equals 78 to

87 per cent breadth of disk; and 49 to 53 per cent of total length. Tail about half the total length. Teeth as in *erinacea*, except more numerous; 66 to 104 rows in each jaw, in 13 specimens, $15\frac{1}{2}$ inches (immature) to 36 inches long. Thorns as in *erinacea*, the large spines along the midline being rarely present except in very young. Colour, light brown above, spotted with round darker patches: with or without one or two large pale eye spots, often black centred,

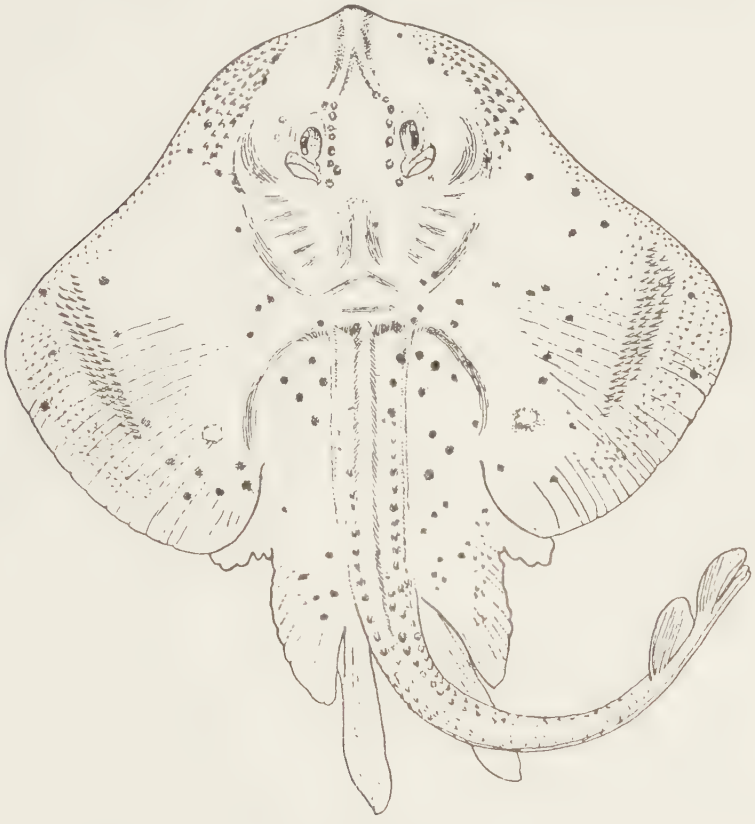


Fig. 22. Spotted skate (*Raja diaphanes*). After Garman.

near rear angle of pectoral. Sides of snout translucent; lower surface white. Maximum length about 3 feet; adults usually 30 to 34 inches.

From near tide mark down to 50 fathoms. Atlantic shelf of North America; southern side of gulf of St. Lawrence and western part of Grand bank, to Chesapeake bay.

R. scabrata Garman. Thorny skate. **2, 4,** 5 and **7** (*R. radiata*). (Fig. 23)

Anterior outline obtuse. Distance to eye in 5 Massachusetts bay specimens one from Halifax, and one from Sable island, $9\frac{1}{2}$ to 31 inches long, equals 29 to 40

per cent of breadth of disk opposite eyes; length of disk from snout to rear angle of pectoral equals 79 to 90 per cent of breadth of disk; and 51 to 59 per cent of total length. Teeth in pavement, in $\frac{42}{40}$ to $\frac{42}{44}$ rows (3 specimens). Twelve to sixteen very large curved thorns with notably stellate bases along midline of tail and disk; a pair on each shoulder; one in front and one behind each eye; one behind each spiracle. Smaller thorns on the snout, in a row flanking the mid-dorsals, on each side of the latter, scattered over the pectorals and disk, and on

Fig. 23. Thorny skate (*Raja scabrata*). After Garman.

the tail. Large smooth bare patches on back behind spiracles, flanking dorsal ridge, and opposite bases of pectorals and of ventrals. Two dorsal fins usually separated by a free space with a thorn. Outer angles of pectorals bluntly angular. Colour, brown above, uniform or clouded: more definitely spotted when young: some with pale spots; white below. Maximum length about 3 feet.

Deeper than 10 to 15 fathoms. Atlantic shelf of North America; Hudson bay, north and east coasts of Newfoundland, the Grand bank and gulf of St. Lawrence to Woods Hole, Mass.; perhaps intergrading with the north European *R. radiata*.

R. radiata Donovan. Starry skate. 3, **4**, **6**, **8**, **9**. (Fig. 24)

Anterior outline obtuse. Distance from snout to eye (in two Norwegian specimens) equals 33 per cent of breadth of disk opposite eyes; length of disk equals 79 to 80 per cent of width of disk, and 54 to 55 per cent of total length. Teeth in 38 to 45 rows. Mid-dorsal thorns as large as in *R. scabrata*, but averaging more numerous; 11 to 19 recorded. Large thorns scattered over body and

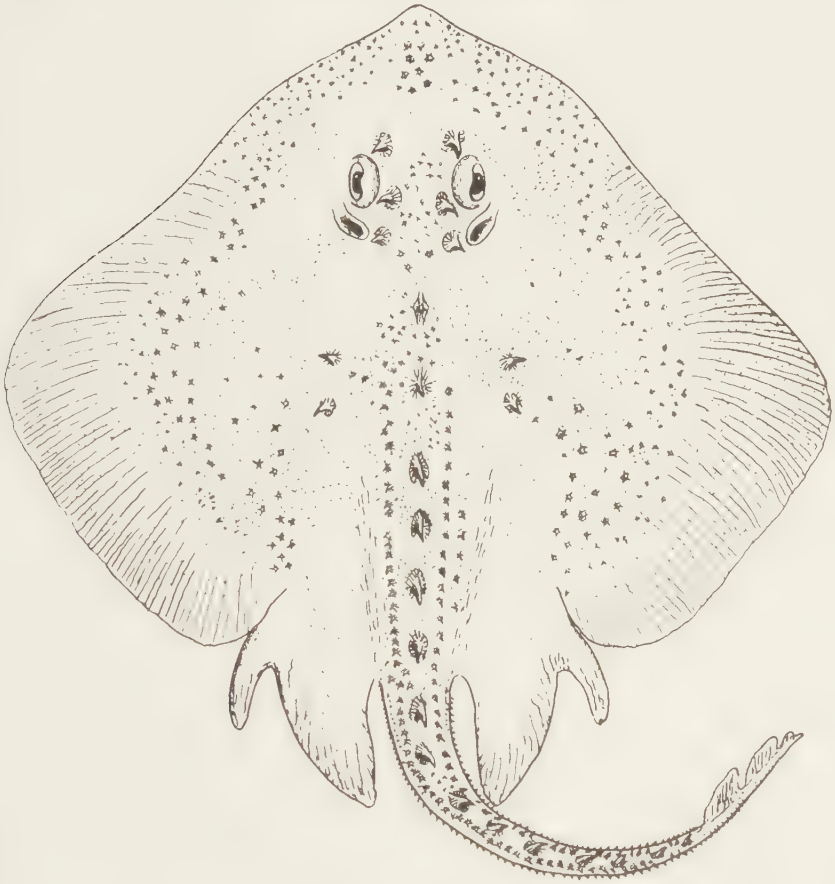


Fig. 24. Starry skate (*Raja radiata*). From Norwegian specimen.

disk, the skin between thorns with tiny spines; outer margin of disk and ventrals almost smooth. Smooth below. Colour, chocolate brown above, with or without paler markings, sometimes irregularly blotched with darker; white below, tinged with pink at margins of fins, and sometimes with brown spots on tail. Maximum reported length 25 inches.

Down to 460 fathoms, usually less than 100. Davis strait, west coast of Greenland, coastal slopes of northeastern Atlantic, from bay of Biscay and North sea north to White sea, Spitzbergen.

R. fyllae* Lütken. 3, **4**, 5, 6, **7**, **9**. (Fig. 25)

This species varies considerably in colour, shape and spination, according to age. Anterior outline of disk obtuse, slightly undulated in young, deeply notched

Fig. 25. *Raja fyllae*. After Lutken.

opposite spiracles in adult male. Width of disk 51 to 56 per cent of total length of fish; length of disk 42 to 47 per cent. Teeth vary with age, 30 to 36 rows in upper jaw, sharp pointed in adults. Upper surface entirely spinulose in young, with bare patches on median and posterior disk and ventrals in adult males. A median row of spines (34 to 37) extends from the shoulder to the first dorsal in young; no spines between dorsals, which are close set. Median series in adult

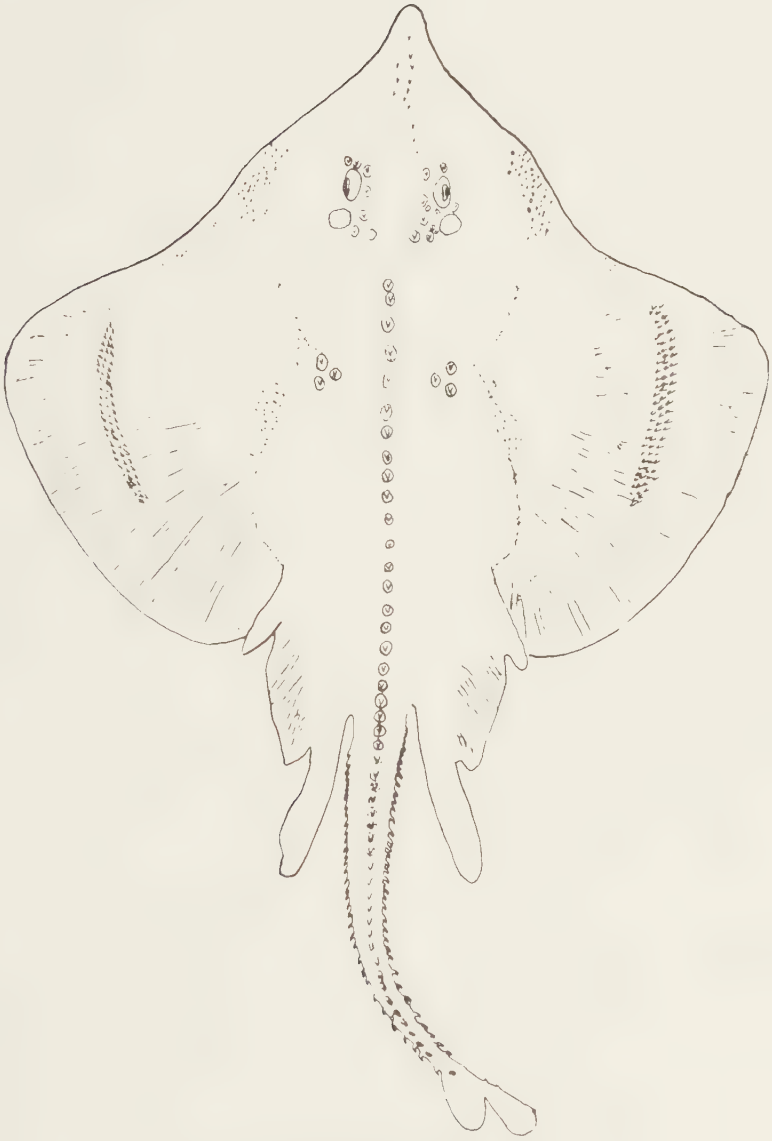


Fig. 26. *Raja lintea*. After Clark.

reduced in number while side rows, inconspicuous in young, are much more pronounced. One or 2 preorbital and 1 or 2 postorbital spines in young with 3 in adult. Adult male with spines along margin of snout and anterior pectoral region with elongate patch usual on pectorals of the male of various species. Smooth below. Colour of adult male uniform ashy gray above, white or grayish brown below. Young brown, with large spots or blotches of darker brown more or less pronounced. Reaches a length of about 22 inches.

From 216 to 983 fathoms. Davis strait, Spitzbergen, Barents sea, northwest Scotland.

R. lintea* Fries. **4, 6, 8, 9.** (Fig. 26)

Anterior outline acute. Distance from snout to centre of eye about 55 to 59 per cent of breadth of disk abreast the eyes; length of disk from snout to rear angle of pectoral equals about 87 to 93 per cent of breadth of disk; breadth of disk 59 to 64 per cent of total length of fish; tail from hind margin of anus slightly less than one-half the total length. Teeth in 47 to 50 rows in each jaw, pointed, with shorter points and broader bases in the female than in the male. Upper surface more or less smooth. A row of 47 to 48 thorns along mid line of back from a little behind spiracles to base of dorsals which are confluent. Several spines immediately in front of and along inner margin of eye with a triangular patch opposite each spiracle; a triangular patch also over each shoulder. Various small spines on rostral process, on outer margin of disk opposite eyes, with a few scattered on other parts of body. A band of coarse spines near outer margins of pectorals in male. Tail has on each side of the median row a marginal series of strongly recurved spines. Lower surface smooth.

Colour slate gray above; under surface white, usually with a broad band of gray around the outer angle and along posterior margin of disk; a patch of gray on each side of vent, on posterior lobe of ventrals; tail spotted or banded with gray. Reaches at least 42 inches in length.

From 80 to 350 fathoms. Davis strait, Iceland, Norwegian coast.

R. spinicauda* Jensen. **6, 9.** (Fig. 27)

Anterior outline nearly a right angle. In two specimens 29 and 41¼ inches long, respectively, distance from snout to centre of eye equals about 53 per cent of breadth of disk abreast the eyes; length of disk from snout to rear angle of pectoral equals about 87 per cent of the breadth of disk; breadth of disk 65 to 70 per cent of total length of fish; tail from hind margin of anus 66 to 69 per cent of total length. Teeth in about 31 or 32 rows in each jaw (one specimen, 55 inches long). Upper surface with numerous small spines, particularly dense along mid line of body and tail; a single row (21-26) of larger spines along mid line of tail. Smooth below. Colour, blue-gray above; white below, with dark

gray spots along posterior margin of disk, on ventrals and tail. Recorded up to 55 inches in length.

From 120 to 440 fathoms. Davis strait, southwest Greenland.

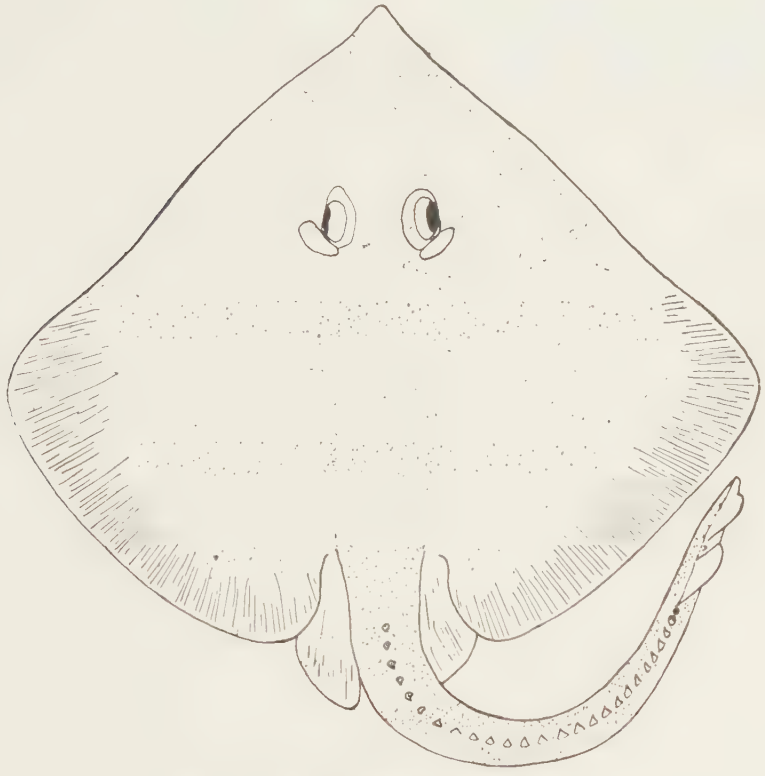


Fig. 27. *Raja spinicauda*. After Jensen.

R. granulata Goode and Bean. Deep water skate. **1**, 4, 5, 7. (Fig. 28)

Anterior outline acute. Disk rhomboidal, anterior part about a right angle; lateral angles acute. Snout to centre of eye equal to 49 per cent breadth of disk opposite eyes, in specimen $33\frac{1}{2}$ inches long. Length of disk 73 per cent of breadth, and 56 per cent of total length. Spiracles close to eyes. Mouth forms an obtuse angle, its width equal to about 46 per cent of breadth of disk opposite outer corners of mouth. Teeth scattered, in about $\frac{56}{56}$ rows, long, pointed, with broad bases. Ventrals deeply notched. Dorsals higher than long: space between them equal to height of the second dorsal. Eight large thorns along mid-dorsal line of disk from shoulders to base of tail; and 5 on the anterior third of tail. Other large thorns before eye (1); behind eye and opposite inner angle of

spiracle (3); and shoulder (2). Numerous small prickles with stellate bases on snout, between eyes, along anterior and posterior parts of disk, and along sides of tail. Naked areas between and behind spiracles, flanking dorsal ridge, and on ventrals. Ventral surface of disk sprinkled sparsely with minute rounded tubercles. Colour, in alcohol, light brown above, darker along margin of fins, pale (probably white) below, but with margins of pectorals dark brown; large dark areas on central part of disk and on ventrals. Maximum size unknown.

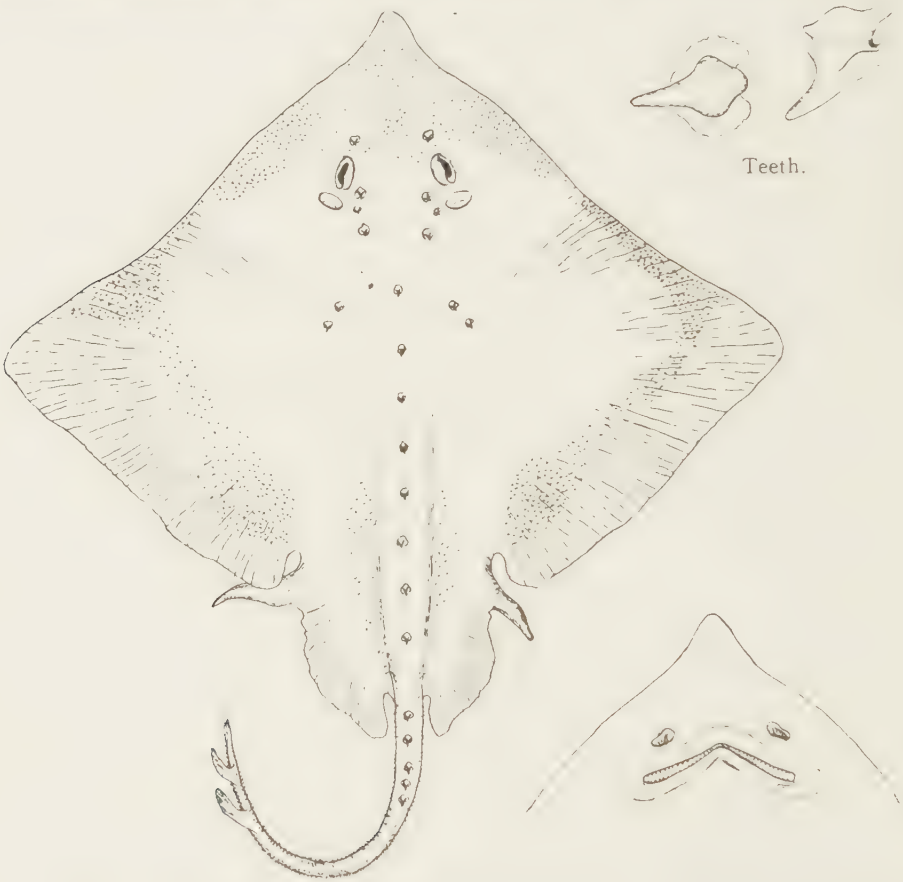


Fig. 28. Deep water skate (*Raja granulata*). From specimen described (p. 29).

From 200 fathoms. La Have bank (type) and continental edge off Halifax, Nova Scotia (lat. 42°37′; long. 62°55′).

R. hyperborea* Collett. Arctic skate. 3, **4**, 6, **8**, **9**. (Fig. 29)

Anterior outline acute. Distance from snout to centre of eye equal to about 50 per cent of breadth of disk opposite eyes; length of tail from cloaca 39 to 46

per cent of total length; large thorns arranged as in the *radiata-scabrata* group, except smaller, their bases less definitely stellate; with a larger number (22-31) in the mid-dorsal line, and usually 3, instead of 2, on each shoulder, and 3 at the inner margin of each orbit. Many smaller prickles between the large thorns, and sides of the tail prickly; lower surface smooth. Teeth scattered, needle-pointed; alike in the two sexes; 40 to 44 rows in upper jaw. Dorsal fins usually

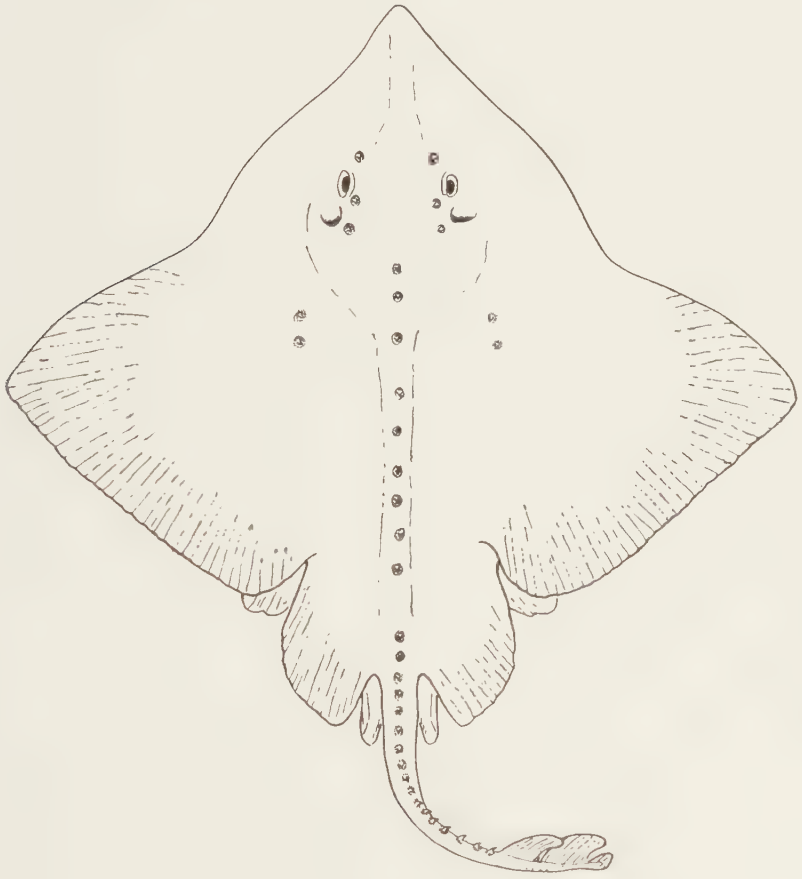


Fig. 29. Arctic skate (*Raja hyperborea*). After Collett.

separated by 1 or 2 thorns. Colour, bluish to brownish gray, or red-brown above, sometimes with light and dark spots; lower surface variegated white and dark. Maximum recorded length about 34 inches.

Down to 1300 fathoms. Arctic: western coast of Greenland, Spitzbergen and north of Russia and south to Faroe channel.

R. eglanteria Bosc. Clear nosed skate. **2, 4, 7.** (Fig. 30)

Anterior outline approximately a right angle; distance from snout to centre of eye equal to 48 to 51 per cent of breadth of disk opposite eyes; length of disk equals 80 to 84 per cent of breadth, and 53 to 56 per cent of total length. Teeth in about $\frac{48}{48}$ rows. Dorsal fins separated by a considerable interval, interspace usually

Fig. 30. Clear nosed skate (*Raja eglanteria*). After Garman.

with one or two thorns. Large thorns confined to mid-dorsal line, to groups opposite and behind eyes, a pair on each shoulder, and to sides of tail. Elsewhere upper surface of disk bears small, very sharp prickles, most thick-set on anterior parts of pectorals, over head and snout, and amid the large thorns on middle of back and along tail. Colour, brown above, variously spotted with round to elongate

darker brown blotches with a large and very conspicuous translucent or white space on either side of snout; lower surface white. Maximum length about 3 feet.

Chiefly shoaler than 15 fathoms. Cape Ann, Massachusetts, to Florida; rare north of Nantucket shoals.

R. senta Garman. Smooth skate. **2, 4,** 5, **7.** (Fig. 31)

Anterior outline roughly a right angle. Distance from snout to centre of

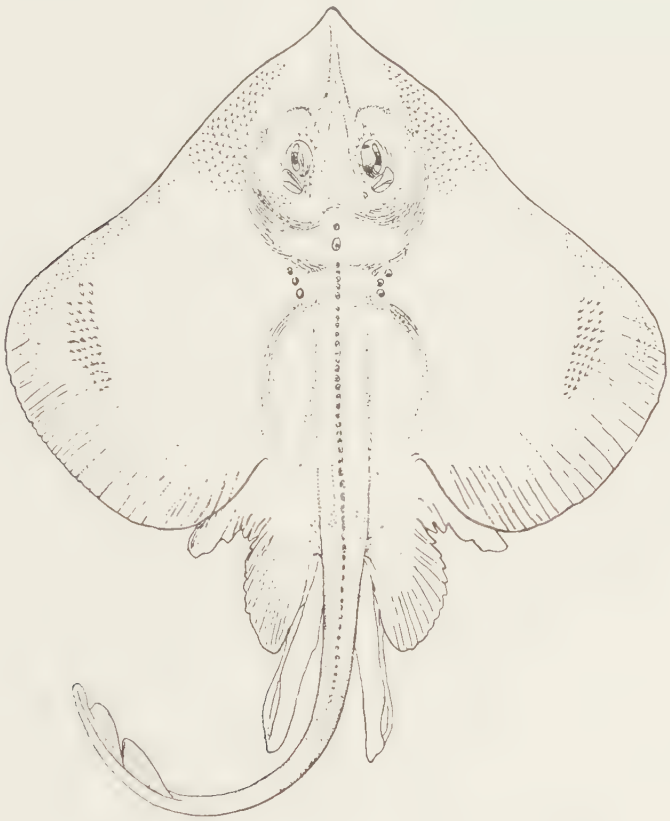


Fig. 31. Smooth skate (*Raja senta*). After Garman.

eye equals about 47 per cent of breadth of disk opposite eyes; length of disk equals 80 to 87 per cent of breadth, and about one-half total length; outer angles rounded. Teeth in about $\frac{38}{38}$ rows. About 30 thorns (some lost) in mid-dorsal row, beginning about midway between eye and shoulder, and extending one-third way back along tail, decreasing in size posteriorly; other large thorns about eye

and on shoulder. Upper surface of disk and ventrals covered almost everywhere with small spines which are somewhat larger anteriorly. Males with a patch of larger spines on outer pectorals. Tail with small spines which extend underneath. No free space between dorsals. Separable from *R. eglanteria* by smoother skin, absence of spots and of mid-dorsal thorns on rear half of tail; from *stabuliforis* by less produced snout, and extension of mid-dorsal thorns forward to shoulders; from *R. granulata* by smooth, white, lower surface, and more numerous mid-dorsal thorns. Colour of upper surface pale brown with numerous rather obscure spots darker than the ground colour, sometimes with white markings. Maximum length 2 feet.

La Have bank; generally distributed in gulf of Maine on hard or soft bottom, on offshore fishing banks and in deeper parts (80 to 100 fathoms). Shoalest capture 25 fathoms.

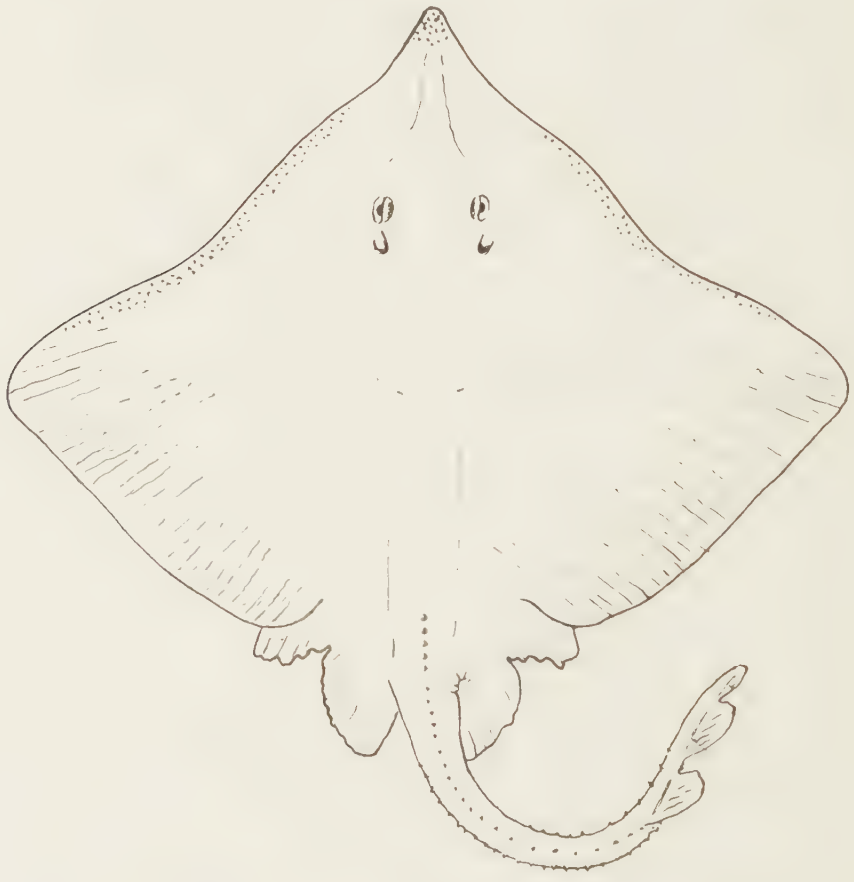


Fig. 32. Barndoor skate (*Raja stabuliforis*). After Garman.

R. stabuliforis Garman. Barndoor skate. **2, 4,** 5 and **7** (*R. laevis*). (Fig. 32)

Anterior outline very acute. Distance from snout to eye (2 specimens, ♂41¾ and ♀47¼ inches long) equals 55 to 59 per cent of breadth of disk opposite eyes; length of disk equals 77 to 78 per cent of breadth of disk and 56 to 73 per cent of total length. Teeth in pavement, in $\frac{32}{32}$ to $\frac{36}{36}$ rows, sharper in males than in females. Snout narrow, projecting; but blunt tipped; outer corners of pectorals angular; disk diamond- or lozenge-shaped; dorsal fins separated by a definite interspace, with one or more spines; tip of caudal extending farther than in other local species. Mid-dorsal thorns along tail and on hinder part of back. Other spines relatively smaller than in other local skates; restricted to sides of tail, tip of snout, bands along front edges of pectorals and anterior margins of disk; in front of and behind eyes; with a few others sparsely scattered. Skin of back between spines smooth. Colour: upper surface brown, usually reddish, variously marked with darker brown blotches or spots, sometimes pale-edged; and often with pale marblings. Lower surface white or pale gray; darker toward snout; spotted with black on abdomen. The largest of local skates; maximum length 6 feet or more.

Tide mark down to more than 100 fathoms; chiefly 5 to 60 fathoms. Continental shelf of eastern North America, southern side of gulf of St. Lawrence and southwestern part of Grand bank to Florida.

NARCACIONTIDAE. TORPEDOES

General form flattened; tail stout, without spines; caudal fin large, triangular; skin perfectly smooth, without spines or prickles; electric organs present.

Genus **NARCACION**

With two dorsal fins; tail shorter than disk, with lateral folds; ventral fins not united.

N. nobilianus (Bonaparte). Torpedo. **2, 4, 7** (*Tetronarce occidentalis*). (Fig. 33)

Disk subcircular, considerably broader than long, its margin evenly rounded, truncate anteriorly. In specimen examined, 31½ inches long; distance from tip of snout to eyes (relatively much smaller than in the skates) only about 20 per cent of breadth opposite eyes; length of disk about equal to 55 per cent of total length; tail about 40 per cent of total length. First dorsal at anterior end of tail, origin in front of rear end of base of ventrals; second dorsal only about half as high as first, its height nearly equal to interspace. Caudal fin large, triangular, with rounded angles. Skin smooth. Colour, dark chocolate brown above, and along lower edges of disk, fins and tail; otherwise white below. Length of adults usually 2 to 5 feet. Maximum recorded weight, about 200 pounds.

Tropical and temperate coastal waters, on both sides of north Atlantic; La Have bank and eastern Maine southward to Cuba along American coast. Occasional north of cape Cod.

Fig. 33. Torpedo (*Narcacion nobilianus*). After Garman.

DASYBATIDAE. STING RAYS

General form flattened, disk-like; tail very slender, with one or more long, serrated, dorsal spines in Atlantic species; no caudal fin; snout not supported by a rostral cartilage; pectorals meeting in front without definite rostral fins. Skull flattened, with eyes on dorsal surface; teeth small, in pavement of many rows. Nasoral grooves present.

Genus **DASYBATUS**

No dorsal or caudal fins. Tail at least as long as disk, with one or more dorsal spines, its end lash-like; disk rhombic, broader than long.

D. centroura (Mitchill). Sting ray. **2** and **4** (*D. marinus*); **7** (*Dasyatis centrura*) (Fig. 34)

Disk quadrate, anterior outline very obtuse, distance from snout to eye in specimen 44 inches long equals 31 per cent of breadth of disk opposite eyes; length of disk 85 per cent of breadth, 30 per cent of total length. Lateral corners of pectorals bluntly angular. Tail more than twice as long as disk, tapering to

Fig. 34. Sting ray (*Dasybatus centroura*). After Garman (*D. marinus*).

a lash-like tip, with a cutaneous keel below, but none above, rounded above behind spines. Caudal spines one to three or more, about one-fifth the way back on tail, erectile, their lateral edges strongly serrate. Skin smooth in young specimens; adults with tubercles scattered over central and posterior dorsal surface of disk, and along tail, increasing in number with growth. Colour, brown above, varying dark to light; white below; tail brown. Maximum recorded length, including tail, about 12 feet.

Western Atlantic from cape Cod and Georges bank to cape Hatteras, mostly southward; also Mediterranean.

HOLOCEPHALI. CHIMAEROIDS

No spiracle; gills fringe-like and free at the tips as in bony fishes; gill clefts enclosed by flaps of skin, with only one external gill opening on each side; only 3 pairs of dental plates, 2 in the upper, 1 in the lower jaw, set edgewise. Tail nearly symmetrical.

CHIMAERIDAE. THE CHIMAERAS

Nose pointed, without definitely elongate, or flap-like proboscis; caudal fin without inferior lobe; claspers of male bifid or trifid.

Genus **CHIMAERA**

Characters of the family.

C. affinis Capello. Chimaera. **2, 5, 7.** (Fig. 35)

Body compressed, tapering. Head moderately pointed; without definite proboscis, but its dorsal profile prolonged into a knob above mouth; forehead of male with an erectile cartilaginous hook, its lower surface armed with prickles. Mouth inferior. Gill openings vertical, low on sides of neck. Dorsal fins separated by short interspace, but connected by ridge of skin (about $1\frac{1}{2}$ inches in

Fig. 35. Chimaera (*Chimaera affinis*).

specimen $3\frac{1}{2}$ feet long). First dorsal triangular, length about equal to distance from snout to rear margin of eye, about as high as long, preceded by a spine, free at tip. Second dorsal less than half as high as first, its margin straight, extending back along whole length of trunk; separated from caudal by a deep notch, but no definite interspace. Caudal lanceolate, terminating in a short filament (filament about $1\frac{1}{2}$ inches in $3\frac{1}{2}$ ft. specimen). No separate anal. Ventrals and pectorals triangular; the latter much the larger, their tips reaching nearly to ventrals. Skin smooth, or slightly prickly. Colour, brown or leaden, above and below. Maximum length about 4 feet.

Chiefly 200 to 1200 fathoms. Grand banks to gulf of Mexico; Portugal.

Report of the year 1933

THE WOODS HOLE OCEANOGRAPHIC INSTITUTION

REPORT FOR THE YEAR
1933

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I

TRUSTEES

(As of December 31, 1933)

To serve until 1937

NEWCOMB CARLTON, New York, N.Y.
THE HYDROGRAPHER, United States Navy Department, for the time being
T. H. MORGAN, California Institute of Technology
R. S. PATTON, U. S. Coast & Geodetic Survey, Washington, D.C.
B. W. ST. CLAIR, West Lynn, Mass.
Lt. Commander EDWARD H. SMITH, U. S. Coast Guard, Woods Hole, Mass.

To serve until 1936

WILLIAM BOWIE, U. S. Coast & Geodetic Survey, Washington, D.C.
A. G. HUNTSMAN, University of Toronto
ALFRED F. LOOMIS, Tuxedo Park, New York
JOHN C. MERRIAM, Carnegie Institution, Washington, D.C.
T. WAYLAND VAUGHAN, Scripps Institution of Oceanography, La Jolla, Calif.

To serve until 1935

ISAIAH BOWMAN, American Geographical Society, New York, N.Y.
E. G. CONKLIN, Princeton University
ALEXANDER FORBES, Harvard Medical School
ROSS G. HARRISON, Yale University
FRANK R. LILLIE, University of Chicago
HARLOW SHAPLEY, Harvard University

To serve until 1934

CHARLES FRANCIS ADAMS, Boston, Mass.
THOMAS BARBOUR, Museum of Comparative Zoölogy, Cambridge, Mass.
BENJAMIN M. DUGGAR, University of Wisconsin
JOHN A. FLEMING, Carnegie Institution, Washington, D.C.
SEWARD D. PROSSER, New York, N.Y.
ELIHU ROOT, JR., New York, N.Y.

Ex officio

HENRY B. BIGELOW, Museum of Comparative Zoölogy, Cambridge, Mass.
LAWRASON RIGGS, JR., New York, N.Y.

OFFICERS

FRANK R. LILLIE, President of the Corporation, University of Chicago
LAWRASON RIGGS, JR., Treasurer, 120 Broadway, New York, N.Y.
HENRY B. BIGELOW, Clerk of the Corporation, Woods Hole, Mass.

II

MEMBERS OF THE CORPORATION

(As of December 31, 1933)

Charles Francis Adams, Boston, Mass.
Thomas Barbour, Museum of Comparative Zoölogy, Cambridge, Mass.
Henry B. Bigelow, Museum of Comparative Zoölogy, Cambridge, Mass.
Isaiah Bowman, American Geographical Society, New York, N.Y.
Newcomb Carlton, New York, N.Y.
E. G. Conklin, Princeton University, Princeton, N.J.
Benjamin M. Duggar, University of Wisconsin, Madison, Wis.
John A. Fleming, Carnegie Institution, Washington, D.C.
Ross G. Harrison, Yale University, New Haven, Conn.
A. G. Huntsman, University of Toronto, Toronto, Canada
The Hydrographer, U. S. Navy Department, for the time being, Washington, D.C.
Frank R. Lillie, University of Chicago, Chicago, Ill.
Alfred F. Loomis, Tuxedo Park, N.Y.
John C. Merriam, Carnegie Institution, Washington, D.C.
T. H. Morgan, California Institute of Technology, Pasadena, Calif.
R. S. Patton, U. S. Coast & Geodetic Survey, Washington, D.C.
Seward D. Prosser, New York, N.Y.
Lawrason Riggs, Jr., New York, N.Y.
Elihu Root, Jr., New York, N.Y.
Harlow Shapley, Harvard University, Cambridge, Mass.
B. W. St. Clair, West Lynn, Mass.
Lt. Commander E. H. Smith, U. S. Coast Guard, Woods Hole, Mass.
T. Wayland Vaughan, Scripps Institution of Oceanography, La Jolla, Calif.

III

REPORT OF THE TREASURER

July 11, 1934.

To the Members and Trustees of the Woods Hole Oceanographic Institution:

Gentlemen:

The accounts of the Treasurer through February 28, 1934, have been audited by Messrs. Seamans, Stetson & Tuttle, certified public accountants, and copies of their reports covering the four fiscal years are on file in the office of the Institution. Following are summaries of the various accounts:

PLANT ACCOUNT

Received from Rockefeller Foundation			$580,100.00
Interest on bank balances less Federal Tax on checks			542.13
Received from Carnegie Corporation			27,000.00
Transfers from Current Funds:			
For the year ending Feb. 28, 1931			5,072.32
For the year ending Feb. 29, 1932			8,386.94
For the year ending Feb. 28, 1933			7,734.21
For the year ending Feb. 28, 1934			2,373.56
Reserve for Depreciation			9,813.75
Atlantis construction		$218,674.47	
Atlantis equipment		26,758.82	
Land		27,072.32	
Laboratory construction		323,213.12	
Laboratory equipment		21,361.93	
Asterias construction & equipment		6,570.55	
Crew Room		557.95	
Books		7,000.00	
Cash Depreciation Fund	$2,813.75		
Securities Depreciation Fund	7,000.00	9,813.75	
		$ 641,022.91	$ 641,022.91

ENDOWMENT FUNDS

There was received from the Rockefeller Foundation for the General Foundation Fund		$1,000,000.00	
Less loss on sale of securities		1,305.50	
Balance March 1, 1933		998,694.50	
Less loss from sale of bonds $2,434.70—70% apportioned to General Endowment		1,704.29	
			$ 996,990.21

Forward		$996,990.21
and from the same source for Endowment for upkeep	$419,419.96	
Less loss on sale of securities	559.50	
Balance March 1, 1933	$418,860.46	
Less loss from sale of bonds $2,434.70—30% apportioned to Endowment for Upkeep	730.41	
		418,130.05
A reserve has been set up for amortization of bond premiums, which now amounts to		1,302.00
making a total of		$1,416,422.26
This is represented by bonds of the cost of	$1,236,866.89	
Preferred stocks at a cost of	179,018.75	
and cash on hand	536.62	$1,416,422.26

A full list of the securities will be found in the Auditors' reports.

Interest and dividends receivable from the Endowment Funds, if all securities pay in full, amount to $62,755.00

During the year 1933–1934, the actual amount received from the Endowment Funds, was $62.869.10

RESERVE FUND

On March 14, 1931, the Institution received an anonymous gift of... $30,000.00 consisting of Cash in the amount of $4,271.50 and the balance in stocks.

This has been treated as a Reserve Fund to be used at the discretion of the Trustees.

GENERAL ACCOUNT

For the period from March 1, 1933 to February 28, 1934:

Income:

From the Rockefeller Foundation for Current Expenses for the year ended Feb. 28, 1934	$50,000.00
From Bingham Oceanographic Foundation of Yale University to apply on expense of Caribbean Voyage	1,500.00
From sale of truck previously donated to the Institution	233.00
Interest and Dividends from Investments	62,869.10
Interest on Bank Balances	13.33
TOTAL INCOME	$114,615.43

Expense:

Upkeep of Plant:

Upkeep of Building and Grounds	$6,501.42	
Upkeep of *Atlantis*	6,082.55	
Upkeep of *Asterias*	239.94	
Insurance	7,605.50	
Reserved for Depreciation	6,000.00	
		$26,429.41

Forward			$ 114,615.43
		26,429.41	
Operating Expenses:			
Operation of *Atlantis*	$28,884.36		
Operation of *Asterias*	805.91		
Scientific Salaries	34,408.22		
Scientific Supplies and Equipment	4,094.13		
Travelling Expense	432.29		
Grant to Bermuda Biological Station for Research	2,000.00		
Publications	3,293.88		
Administration	8,357.06		
Miscellaneous Expense	89.23		
Amortization of Bond premiums	434.00	82,799.08	
TOTAL EXPENSE		109,228.49	
Expenditure for Permanent Plant Additions:			
Additions to Laboratory Equipment	$606.69		
Additions to *Atlantis* Equipment	766.87		
Additions to Library	1,000.00	2,373.56	
TOTAL EXPENDITURE			111,602.05
EXCESS OF INCOME			$ 3,013.38

The situation on March 1, 1934, the beginning of the present fiscal year, was as follows:

Current Assets		*Current Liabilities*		
Cash	$5,539,74	Accounts payable:		
Accounts receivable	126.07	To Sundry Creditors	$ 474.09	
		To Depreciation Fund	2,224.17	
				$2,698.26
		Accrued Payroll		885.00
		Current Surplus and Reserve For "Working Balance:"		
		Reserve for Working Balance	$5,000.00	
		Current Surplus (Deficit)	2,917.45	
				2,082.55
	$5,665.81			$5,665.81

Respectfully submitted,
LAWRASON RIGGS, JR.
Treasurer.

IV

FOURTH ANNUAL REPORT OF THE DIRECTOR FOR THE YEAR 1933

BY HENRY B. BIGELOW

I

Equipment

The need of sonic sounding apparatus for "Atlantis" was mentioned in my last report. After investigating the merits of different types, the "Fathometer," manufactured by the Submarine Signal Company, was finally selected, and installed on "Atlantis" last November. The cost, $6,948, was paid from current income. This machine has given satisfaction, for soundings as deep as 3000 fathoms.

During the autumn, radio transmitting and receiving equipment, received as a loan from the United States Coast Guard, was installed. This equipment, which permits transmission and reception on the intermediate commercial frequencies, has already proven its value in making it possible to obtain radio bearings from shore stations, as well as in maintaining communication between ship and shore. The Institution is greatly indebted to the Coast Guard for the loan of this equipment.

A carbon-dioxide fire-extinguishing system, not mentioned in the report for 1932, was installed in June of that year. The cost ($1,080) was paid from the balance of our original construction fund.

Minor improvements on "Atlantis" are:—installation of sinks with running fresh water in upper and lower laboratories and of electric pumps and other fittings for running salt water, for the upper laboratory; additional laboratory furniture; alteration of alcove off mess room to serve as radio-room; new refrigerating coils; new burner for steam heating system; a trawling boom fitted to the mizzenmast; and another dory purchased. An improved type of closing net for horizontal plankton hauls at great depths, and usable in series, was also developed, as a result of experiments carried out at sea during the summer.

No important alterations have been made to "Asterias;" a new skiff was purchased, as her tender.

In the laboratory a low concrete wall was laid, in the aquarium room to retain the overflow from the large concrete tanks. This is

covered by a wooden platform, making access easier to the tanks. An improved device has been installed for holding the water level at the desired height. And three large wooden tanks have been added.

The end of one of the dark rooms has been converted into a kitchenette, with sink and shelves, convenient to the reading room, for use at staff meetings, and for the convenience of workers spending the winter at the laboratory.

A large chart of the North Atlantic, on which to plot the cruises of "Atlantis" has been prepared, for installation on one wall of the chart room.

In June the motor truck was sold for a net price of $200.

We have received the following instruments from Norway, from the Wilkins-Ellsworth Trans-Arctic Submarine Expedition, which, having been purchased by grant made to the latter in September, 1930, are now our property:—10 "Nansen" reversing water bottles; 1 "Meteor" bottom sampler; 1 dynamometer; 13 reversing deep sea thermometers. It is agreed that they are to be returned to Captain Wilkins should he be able to organize a second Arctic Submarine Expedition in the reasonably near future. Such of the "Nansen" water bottles and reversing thermometers as still remained in Norway have been loaned to Dr. H. U. Sverdrup for the Norwegian Circum-Antarctic Expedition. And the "Meinesz" machine for the measurement of gravity, used on the "Nautilus," has been donated to the Geophysical Institute of Bergen, there being no arrangement under which this apparatus (for which we have no direct need) could be imported into the United States, duty free.

The following equipment has been loaned to us:—a dredge from the Marine Biological Laboratory; tide gauge, from the U. S. Coast and Geodetic Survey; three unprotected deep sea thermometers, from the Smithsonian Institution, through the kindness of Dr. Paul Bartsch; a miscellaneous lot of dredge and trawl frames, anchors, and other gear, from the U. S. Bureau of Fisheries; and a deep sea pressure gauge from Dr. William Beebe. We are also indebted to the U. S. Hydrographic Office, and to the U. S. Coast and Geodetic Survey, for charts.

We have received as gifts, a bust and photograph of the famous oceanographer Maury from Mr. Charles R. Crane; a copy of Maury's "Physical Geography of the Sea" from Mr. Francis N. Balch; and, from Professor Norris W. Rakestraw, the chair used by the Prince of Monaco on his research ship "Hirondelle."

During the year "Atlantis" has experienced the following minor

accidents:—her main truck was twice struck by lightning, necessitating slight repairs (the cost being paid by the underwriters) and the installation of a protective device. She also touched bottom in Woods Hole Harbor, but suffered no damage.

II

Library

Contribution to the library of the Marine Biological Laboratory was the same as last year—$1,000; and the library has transferred its oceanographic books to our reading room.

III

Personnel

No change has been made in the administrative staff, or among the officers of "Atlantis."

Captain Terence Keogh had charge of "Asterias" from June until August 1st, when he resigned. During the remainder of her working season, she was in charge of Mr. E. L. Ayers.

In April, as I was to be absent in Europe, Professor Redfield was given charge of the general management of the laboratory, Mr. Iselin of the operations of our two vessels. And this arrangement proved so successful that it was continued.

Changes in the scientific staff of the Institution during the year are as follows:—

Professor A. E. Parr, Curator of the Bingham Oceanographic Collection, Yale University, was appointed Research Associate in Oceanography in November, for one year from February 1, 1933.

On the other hand Mr. Olav Mosby's appointment as Research Associate in Oceanography expired on February 28, 1933. And as he has returned to Norway, severing his connection with the U. S. Coast Guard, this appointment was not renewed. Mr. Floyd M. Soule, who was appointed as his successor on the International Ice Patrol, has succeeded Mr. Mosby on our staff.

Dr. Cornelia L. Carey of Barnard College was appointed Research Associate in Marine Bacteriology, for one year from September 1, 1933.

Dr. Herbert W. Reuszer, Junior Marine Bacteriologist, resigned on September 15, 1933, to take a position elsewhere and Mr. Charles E. Renn has been appointed Assistant in Marine Bacteriology.

In 1930–1931, and again in 1931–1932, appropriation was made

for short-term appointments for "Research Assistants" as explained in previous reports. The title of these appointments has been changed to "Fellowships," for which the budget for the current year included an item of $4,000. These were divided into classes, one for undergraduates, one for graduate students, stipends to be at the rate of $60 per month for the former, and from $85 to $125 per month for the latter. Appointments are made ordinarily for the summer months, at Woods Hole. Thirty-five applications were received, and the following sixteen appointments were made:—Eliot F. Beach, Trygve Braarud, Phil E. Church, Dr. Ancel B. Keys, Benjamin B. Leavitt, Henry Mahncke, Dr. Robert H. Oster, C. E. Renn, Dr. Raymond W. Root, Marshall Schalk, Dr. Mary Sears, Homer P. Smith, Carola F. Whitman, Dr. E. E. Watson, Charles L. Wheeler, Gladys Seiwell.

In addition to staff members and holders of Fellowships, the following investigators worked at the laboratory during the year:—Dr. J. Bjerknes, Mr. Trygve Braarud, Dr. W. F. Bruce, Dr. Cornelia L. Carey, Mr. Gardner Emmons, Dr. C. J. Fish, Dr. Arda Green, Dr. Margaret Hotchkiss, Dr. Martin Johnson, Dr. August Krogh, Dr. Marie Krogh, Dr. G. H. Parker, Dr. Theodor Svedberg, Dr. Roderick Macdonald, Mr. Floyd M. Soule, Dr. Abby H. Turner, Dr. E. E. Watson, and Dr. C. B. Wilson.

Dr. August Krogh, Professor of Zoöphysiology, at the University of Copenhagen, visited the Institution by invitation, under item provided in the budget.

The list also includes three laboratory technicians, and an assistant.

Staff, visiting investigators and holders of Fellowships, represented the following twenty-two institutions:—Barnard College, Bingham Oceanographic Foundation of Yale University, Brown University, Buffalo Museum of Sciences, Cambridge University, University of Copenhagen, Carnegie Institution of Washington, Clark University, College of the City of New York, Flower Medical College, Geophysical Institute of Bergen, Harvard University, International Ice Patrol, International Passamaquoddy Commission, Massachusetts Institute of Technology, New Jersey Agricultural Experiment Station, University of Oslo, Queen's University, Radcliffe College, Rockefeller Institute, Rutgers University, and University of Upsala.

IV

Foreign Travel by the Staff

Prof. Rossby was in Europe from December until May. Most of this time he spent at the Geophysical Institute, Bergen, working on

the theory of turbulent layers in the atmosphere and in the ocean. He also made short visits to Professor V. W. Ekman's Laboratory in Lund, and to the Geophysical Institute in Leipzig.

Dr. Waksman sailed on July 28, for Copenhagen, to preside at a meeting of the Section of Soil Microbiology of the International Society of Soil Science. Before his return, in October, he visited various laboratories in Germany, the Scandinavian countries, France and Italy.

Mr. Seiwell, under a Fellowship in Oceanography from the Scandinavian American Foundation, sailed on October 12th, 1932, for Göteborg, Sweden, then after a short time in Oslo, proceeded to Bergen where he spend the winter of 1932–1933 at the Geophysical Institute working up the "Atlantis" data on the distribution of oxygen and of phosphates in the North Atlantic. During the spring and summer, he paid short visits to Professor Hjort's Laboratory in Oslo, to Paris, to the Plymouth Laboratory, to London and to the Institut für Meereskunde in Berlin. He also took part on a short cruise with Dr. Helland-Hansen on the "Armauer Hansen." He returned to Woods Hole on September 4th.

I myself attended the meeting of the International Council for the Exploration of the Sea, in Paris in May.

V

Publication

The first number of the new serial, "Papers in Physical Oceanography and Meteorology," appeared in March, 1933, and six oceanographic contributions have been published in this serial up to December 31, 1933.

We have so far been able to place papers, by our staff, on chemical and biological subjects, in other periodicals—several of them in the Biological Bulletin.

Contributions from the Institution are numbered serially. And we plan to bind these in sets, for annual distribution.

A report of the Institution for the years 1930–1932 was printed separately. And the physical and chemical data, for "Atlantis" stations, 1930–1932 were transmitted to the International Council for the Exploration of the Sea, for inclusion in their Bulletin Hydrographique for the year 1932, published in 1933.

The following numbered contributions, from the Institution, appeared during the year:

No. 1. Sverdrup, H. U. Scientific Results of the *Nautilus* Expedition, 1931 under the command of Capt. Sir Hubert Wilkins. Part I. Introduction and nar-

rative. Papers in Physical Oceanogr. and Meteorol., Vol. II, No. 1. March, 1933. pp. 1–15.

No. 2. Sverdrup, H. U. Scientific Results of the *Nautilus* Expedition, 1931 under the command of Capt. Sir Hubert Wilkins. Part II. Oceanography. Papers in Physical Oceanogr. and Meteorol., Vol. II, No. 1. March, 1933. pp. 16–63.

No. 3. Soule, Floyd M. Scientific Results of the *Nautilus* Expedition, 1931 under the command of Capt. Sir Hubert Wilkins. Part III. Echo-sounding. Papers in Physical Oceanogr. and Meteorol. Vol. II, No. 1. March, 1933. pp. 64–76.

No. 4. Villinger, B. Scientific Results of the *Nautilus* Expedition, 1931 under the command of Capt. Sir Hubert Wilkins. Part IV. Schweremessungen auf der "Nautilus" Expedition, 1931. Papers in Physical Oceanogr. and Meteorol. Vol. II, No. 3. June, 1933. pp. 1–16.

No. 5. Stetson, Henry C. Scientific Results of the *Nautilus* Expedition, 1931 under the command of Capt. Sir Hubert Wilkins. Part V. The bottom deposits. Papers in Physical Oceanogr. and Meteorol. Vol. II, No. 3 June, 1933. pp. 17–37.

No. 6. Waksman, Selman A. and Cornelia L. Carey. Rôle of Bacteria in the Decomposition of Plant and Animal Residues in the Ocean. Proc. Soc. Exper. Biology and Medicine. Vol. 30, 1933. pp. 526–527.

No. 7. Green, Arda A., and Raymond W. Root. The equilibrium between hemoglobin and oxygen in the blood of certain fishes. Biol. Bull., Vol. 64, 1933. pp. 383–398.

No. 8. Rakestraw, Norris W. Studies on the biology and chemistry of the Gulf of Maine. I. Chemistry of the waters of the Gulf of Maine in August, 1932. Biol. Bull., Vol. 64, 1933. pp. 149–158.

No. 9. Gran, H. H. Studies on the biology and chemistry of the Gulf of Maine. II. Distribution of phytoplankton in August, 1932. Biol. Bull., Vol. 64, 1933. pp. 159–182.

No. 10. Waksman, Selman A., H. W. Reuszer, Cornelia L. Carey, Margaret Hotchkiss, and C. E. Renn. Studies on the biology and chemistry of the Gulf of Maine. III. Bacteriological Investigations of the Sea Water and Marine Bottoms. Biol. Bull., Vol. 64, 1933. pp. 183–205.

No. 11. Green, Arda A., and Alfred C. Redfield. On the respiratory function of the blood of the porpoise. Biol. Bull., Vol. 64, 1933. pp. 44–52.

No. 12. Waksman, Selman A. On the distribution of organic matter in the sea bottom and the chemical nature and origin of marine humus. Soil Sci., Vol. 36, 1933. pp. 125–147.

No. 13. Redfield, Alfred C. The Evolution of the Respiratory Function of the Blood. Quart. Rev. Biol., Vol. 8, 1933. pp. 31–57.

No. 14. Iselin, C. O'D., II. Some phases of modern deep sea oceanography. General appendix to the Smithsonian Report for 1932. 1933. pp. 251–267, 4 pls.

No. 15. Harwood, E. Monroe, and C. F. Brooks. Monthly sequence of Surface Temperature on the New York-San Juan Steamship route. Trans. Amer. Geophys. Union, Fourteenth Ann. meeting, 1933. pp. 173–80.

No.16. Redfield, Alfred C., and Elizabeth N. Ingalls. The oxygen dissociation curves, of some bloods containing hemocyanin. Journ. Cell. and Comp. Physiol., Vol. 3, 1933. pp. 169–202.

No. 17. Waksman, Selman A., Cornelia L. Carey, and Herbert W. Reuszer. Marine Bacteria and their rôle in the Cycle of Life in the Sea. I. Decomposition of marine plant and animal residues by bacteria. Biol. Bull., Vol. 65, 1933. pp. 57–79.

No. 18. Waksman, Selman, A., Margaret Hotchkiss, and Cornelia L. Carey. Marine Bacteria and their rôle in the Cycle of Life in the Sea. II. Bacteria Concerned in the Cycle of Nitrogen in the Sea. Biol. Bull., Vol. 65, 1933. pp. 137–167.

No. 19. Reuszer, Herbert W. Marine Bacteria and their rôle in the Cycle of Life in the Sea. III. The Distribution of Bacteria in the Ocean Waters and Muds About Cape Cod. Biol. Bull., Vol. 65, 1933. pp. 480–497.

No. 20. Colman, John. The Wood's Hole Oceanographic Institution: Sonic Soundings, and an account of a Winter Cruise to the Somers Islands. Geographical Journ., Vol. 82, 1933. pp. 326–336.

No. 21. Welsh, John H. Light intensity and the extent of activity of locomotor muscles as opposed to cilia. Biol. Bull., Vol. 65, 1933. pp. 168–174.

No. 26. Clarke, George L. Observations on the penetration of daylight into mid-Atlantic and coastal waters. Biol. Bull., Vol. 65, 1933. pp. 317–337.

No. 27. Iselin, C. O'D. The development of our conception of the Gulf Stream system. Trans. Amer. Geophys. Union, Fourteenth Ann. meeting, 1933. pp. 226–231.

No. 29. Rakestraw, Norris W. and Frank B. Lutz. Arsenic in sea water. Biol. Bull., Vol. 65, 1933. pp. 397–401.

No. 31. Mitchell, Philip H. and Norris W. Rakestraw. The buffer capacity of sea water. Biol. Bull., Vol. 65, 1933. pp. 437–451.

No. 33. Clarke, George L. Diurnal migration of plankton in the Gulf of Maine and its correlation with changes in submarine irradiation. Biol. Bull., Vol. 65, 1933. pp. 402–436.

No. 34. Bigelow, Henry B. Studies on the Waters of the Continental Shelf, Cape Cod to Chesapeake Bay. I. The Cycle of Temperature. Papers in Physical Oceanogr. and Meteorol. Vol. II, No. 4, 1933. pp. 1–136.

No. 37. Parker, George H. The color changes of elasmobranch fishes. Proc. Nat. Acad. Sci., Vol. 19, 1933. pp. 1038–1039.

VI

Coöperation

By vote of the Executive Committee, September 8, 1932, the director was authorized "to enter into consultation with the Bingham Oceanographic Foundation of Yale University, with a view to establishing coöperative relations for the investigation of the Caribbean-Gulf of Mexico region." Pursuant to this vote, Professor A. E. Parr, Curator of the Bingham Oceanographic Collection, Yale University, was appointed a member of our staff in November, as noted above. Plans were worked out, for a general oceanographic survey of the Caribbean, which was carried out on "Atlantis," as described below, under the heading "Scientific Program." The Foundation contributed $1,500 toward the cost of the expedition, and Prof. Parr acted as scientist in charge.

Other important items of coöperation are as follows:—

On the June cruise to the Gulf of Maine "Atlantis" worked chiefly on the distribution of eggs and young of the haddock, for the U. S.

Bureau of Fisheries, Mr. Wm. C. Herrington of the Bureau acting as scientist in charge.

We were able to assist in the preparations for a survey cruise by the U.S.S. "Hannibal," in the Panamic region under the direction of the U. S. Hydrographic Office, by taking Commander C. C. Slayton on an "Atlantis" cruise in September for instruction in oceanographic technique. And Dr. Paul Bartsch, of the U.S. National Museum, also made a study of the equipment on "Atlantis" in connection with a cruise to the West Indies, on the steam yacht "Caroline," carried out under the auspices of the Smithsonian Institution.

Laboratory accommodations were placed at the disposal of four of the members of the International Passamaquoddy Commission, and their secretary, during the winter. And similar arrangements have been made for the Civilian Oceanographer of the International Ice Patrol; also for Lt. Commander Smith, to work on the scientific aspects of the Patrol.

The tide gauge, originally lent us by the U. S. Coast and Geodetic Survey, is being in continuous operation at the request of the Survey, to whom the monthly marigrams are sent.

All temperatures taken by "Atlantis" between 300 meters and the surface, to the north of Latitude 30° are being supplied to Dr. Harold Thompson, Director of the Newfoundland Fisheries Research Laboratory, for use in connection with his studies of seasonal and annual fluctuations in the waters around Newfoundland.

Surface temperature and salinity data are being sent to the Institute für Meereskunde in Berlin.

All soundings, taken in depths greater than 300 fathoms, are being forwarded to the International Hydrographic Bureau, Monaco, for inclusion in the new Bathymetric Chart of the Oceans. And locations of all "Atlantis" stations are supplied to the U. S. Hydrographic Office.

VII

Field Work

During the year "Atlantis" has made the following cruises:—

Cruise 15: February to May; Cape Hatteras to Bermuda, Bermuda to Nassau, Nassau to Anegada Passage; detailed physical survey along profiles of the Caribbean Sea; five coastal profiles between Florida and Cape Hatteras.

Cruise 16: June–July; Survey of Gulf of Maine (southern half) and Haddock investigations.

Cruise 17: July; Survey of Gulf of Maine (northern half).

Cruise 18: July; Simultaneous horizontal plankton hauls with closing nets and light intensity measurements in Gulf of Maine.

Cruise 19: July; South from Woods Hole across Gulf Stream, for physical observations, deep-water and bottom samples, and closing net hauls.

Cruise 20: August; South into deep water, for study of vertical distribution of plankton with horizontal closing nets.

Cruise 21: September; Survey of the Gulf of Maine.

Cruise 22: October; Survey of the Gulf of Maine.

Cruise 23: December; Survey of the Gulf of Maine.

Summary of observations

Total distance sailed	17,605 miles
Number of days at sea	198
Number of deep stations	158
Number of shallow stations	290
Temperature and salinity observations	5,357
Oxygen determinations	4,815
Samples for NO_2 and NO_3	1,500
Alkalinity measurements	1,750
Samples for Phosphate phosphorus	2,000
Tows for deep sea fish	6
Horizontal tows with closing plankton nets	166
Oblique plankton hauls	70
Vertical plankton hauls (Helgoland net)	51
Phytoplankton samples	378
Mud cores	18
Bottom samples	135
Sonic soundings (deep)	741
Evaporation measurements	90 days

"Asterias" as in past years has been used for the various lines of work that are carried on near Woods Hole, in the inshore waters, chiefly in connection with Dr. Waksman's, Dr. Redfield's, Dr. Rakestraw's and Mr. Stetson's studies.

VIII

Scientific Program

The major projects on which the members of the Institution are engaged are listed in the annual announcement, and reports of progress by the members of the staff appear below, in the Appendix.

During March and April "Atlantis," Professor Parr in charge, carried out a general survey (128 stations) of the Caribbean Sea. The principal passages between the Antillean chain were sounded, and blocked off by careful hydrographic sections. Sufficient chemical data were obtained to give a clear picture of the distribution of oxygen, phosphates, nitrates, nitrites and alkalinity. This survey, combined with work already done by the Bingham Oceanographic Foundation, can be used as the basis for subsequent work in the West Indian region.

A systematic investigation of the dynamic circulation and biological chemistry of the waters of the Gulf of Maine has also been commenced, with cruises to the Gulf in June, September, October and December.

Other important investigations (not mentioned in the announcement) were by Dr. August Krogh, on the organic content of ocean waters and sea bottoms, and by the members of the International Passamaquoddy Commission on plankton distribution and hydrography of the Bay of Fundy in relation to the Gulf of Maine. A study of the distribution of bathypelagic animals in the offing of Woods Hole has also been started, to be continued, on "Atlantis" cruises, as opportunity offers.

V

STAFF

(As of December 31, 1933)

Henry B. Bigelow, Professor of Zoölogy, Harvard University, Director
Cornelia L. Carey, Barnard College, Research Associate in Marine Bacteriology
George L. Clarke, Tutor and Instructor, Harvard University, Junior Biologist
Columbus Iselin, Assistant Curator of Oceanography, Museum of Comparative Zoölogy, Research Associate in Physical Oceanography
A. E. Parr, Curator of the Bingham Oceanographic Collection, Yale University, Research Associate in Oceanography
Norris W. Rakestraw, Associate Professor of Chemistry, Brown University, Research Associate in Physical Chemistry
Alfred C. Redfield, Professor of Physiology, Harvard University, Senior Biologist
C. E. Renn, Rutgers University, Assistant in Marine Bacteriology
C. G. Rossby, Professor of Meteorology, Massachusetts Institute of Technology, Oceanographer
H. R. Seiwell, Investigator in Oceanography
Floyd M. Soule, Senior Physical Oceanographer, U. S. Coast Guard, Research Associate in Oceanography
Henry C. Stetson, Assistant Curator of Paleontology, Museum of Comparative Zoölogy, Research Associate in Submarine Geology
Selman A. Waksman, Microbiologist, New Jersey Agricultural Experiment Station, Marine Bacteriologist
Captain Sir Hubert Wilkins, Research Associate in Oceanography

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William C. Schroeder, Business Manager
Miss Virginia B. Walker, Secretary and Administrative Assistant
William Schroeder, Superintendent of Buildings and Grounds

APPENDIX

Reports of progress by Investigators working at the Institution during 1933.

PHYSICAL OCEANOGRAPHY OF THE WATERS ON THE CONTINENTAL SHELF, CAPE COD TO CHESAPEAKE BAY

HENRY B. BIGELOW

The account of the Cycle of Temperature, mentioned in last year's report, was completed, with the assistance of Dr. Mary Sears, and has been published, as Contribution No. 34 of the Institution.

INVESTIGATIONS OF PHYTOPLANKTON

TRYGVE BRAARUD

1. About 100 samples of sea water, collected during the work of the International Passamaquoddy Fisheries Commission for quantitative studies of the microplankton of the Bay of Fundy and the Gulf of Maine, were examined by means of centrifuging and subsequent counting. These samples very clearly brought out the minimum in the occurrence of phytoplankton during the month of June which previously had been observed at some stations in the Bay of Fundy region and which is perhaps the most striking feature in the plankton conditions of this region. The causes of this phenomenon are still obscure, but there seems to be some evidence that changes in the stability of the upper water layers in connection with the relatively low light-intensity of the Fundy region may mostly account for it. A large number of illustrations for the final report on the phytoplankton part of the Passamaquoddy investigations were prepared during the summer.

The content of microplankton, in twenty samples, from two stations in the Gulf of Maine water, collected in July, during Dr. George Clarke's investigations on the vertical migrations of zoöplankton, was examined in the same way as those mentioned above. Compared with the only quantitative data available on the phytoplankton conditions of the Gulf during summer time, viz. those published by Dr. H. H. Gran for 1932, these data give indication of fairly large variations at the same locality from one year to another.

STUDIES OF THE DISTRIBUTION OF SURFACE TEMPERATURE IN THE WESTERN NORTH ATLANTIC, BASED ON THERMOGRAPH RECORDS

PHIL E. CHURCH

Twenty synoptic charts were completed in addition to the seventy previously made. The charts now include the period from January 1929 to April 1933. The sea has been further extended to cover all of the North Atlantic west from a line between Halifax–Cape San Roque, including portions of the Gulf of Mexico and the Caribbean Sea. Tabulation of the mean temperatures by weeks for each degree of latitude for six steamship routes was completed for the period of the charts. For January and February, 1933, weekly charts were constructed in the attempt to discover the trajectory of Gulf Stream water which invaded the Slope water area. These charts are being compared with the wind data to see if there is any correlation between surface water movements and wind directions and force.

A report on the surface temperature characteristics for the western North Atlantic is being prepared.

DIURNAL MIGRATION OF ZOÖPLANKTON IN RELATION TO THE PENETRATION OF LIGHT, AND STUDIES ON THE PHYSIOLOGY OF COPEPODS

GEORGE L. CLARKE

The investigation of the diurnal migration of copepods was continued from the "Atlantis," using the five simultaneous closing nets. Plankton hauls were made at frequent intervals at a 54-hour station in the deep water of the Gulf of Maine and at a 24-hour station on Georges Bank. At the same time measurements were made of the penetration of the blue component of daylight into the sea using our photo-electric photometers. (The red component was measured, also, by Dr. R. H. Oster.) Samples taken for the determination of the vertical distribution of phytoplankton were analyzed by Mr. Trygve Braarud. The following additional factors were measured at both stations: temperature, salinity, pH, nitrites, nitrates, phosphates, and oxygen. At the first station nineteen series of measurements of the temperature at 5 meter intervals down to 50 meters were made under the direction of Prof. C. G. Rossby. A pronounced change in temperature every few hours was revealed, the variations showing a distinct tidal rhythm.

In the laboratory, work was continued on the development of a

suitable technique for raising copepods in persistent cultures in order that their physiological responses may be accurately studied under controlled conditions. Quantities of green flagellates and diatoms were grown to serve as food. The survival of the copepods in the laboratory was improved, certain individuals living over a month.

Mr. Charles Wheeler, as research assistant, carried out a qualitative and quantitative analysis of the plankton catches made on the "Atlantis." In addition, measurements were made of the lengths of several hundred copepods from various depths. And Mr. Lucius Wing made a study of the mating habits of certain of the free-swimming copepods.

THE BIOLOGY OF ZOÖPLANKTON IN THE GULF OF MAINE AND BAY OF FUNDY WITH SPECIAL REFERENCE TO PRODUCTION AND DISPERSAL

C. J. FISH

The present studies form a part of an oceanographical program sponsored by the International Passamaquoddy Fisheries Commission. Field investigations covered the period from July 1931 to September 1932, and subsequent laboratory work has been carried on at Woods Hole. During the past year physical and biological analyses have been completed and a report is in the process of preparation.

To date the following results have been described:

I. Physical hydrography:

a) The annual temperature cycle based on, 1) daily readings at St. Andrews, Eastport and Portland, supplemented by monthly readings at three other significant points in the Bay of Fundy; 2) the mean annual temperature cycle at St. Andrews for the period 1922–1931; 3) The annual cycle for the warmest (1931) and the coldest (1923) years during this period.

b) The horizontal distribution of temperature and salinity based on projections at different levels for each cruise.

c) The surface circulation and rate of drift during the period of vernal augmentation of zoöplankton as indicated by 106 returns from 446 drift bottles set out in the Gulf and Bay in May and June, 1932.

II. Volume of zoöplankton:

a) The quantitative distribution of the population in August–September 1931, and from April to September in 1932.

b) The annual fluctuation in the volume of zoöplankton at three stations in the Bay of Fundy.

III. Biology of zoöplankton:

a) The biology of the following species endemic in the Gulf of Maine, including breeding periods, number of annual broods, rate of growth, successful and unsuccessful production areas, dispersal, and correlated physical controlling factors:— *Meganyctiphanes norvegica*, *Thsyanoessa inermis*, *T. neglecta*, *T. longicaudata*, *T. raschii*, *Calanus finmarchicus*, and *Pseudocalanus elongatus*.

INVESTIGATION OF SEA SURFACE TEMPERATURES IN THE WESTERN NORTH ATLANTIC

E. MONROE HARWOOD

In the study of sea surface temperatures in the western North Atlantic, and their relation, if any, to the contemporary weather conditions on the Atlantic coast, work was carried on at Woods Hole from July 5th to August 5th, 1933, with the aid of a gift to Blue Hill Observatory by Mr. Arthur Rotch, of Milton. In coöperation with Phil E. Church, revisions were made in previously drawn synoptic charts on the strength of new data, new charts were drawn to March 1933 and the average sea surface temperature for each degree of latitude along each steamship route was tabulated for each available week.

From August to December the weekly sea surface temperatures were reduced to periods of months. The mean monthly air temperatures and the total precipitation for several Atlantic coast stations were also tabulated. The material is ready for graphing and comparison.

STUDIES BASED ON "ATLANTIS" TEMPERATURE AND SALINITY OBSERVATIONS

COLUMBUS ISELIN

a) Distribution of Temperature and Salinity in the Western North Atlantic.

The studies in this field, begun last year, are now in manuscript form. There remain to be added the results from last winter's Bermu-

da-Nassau section and from the four coastal lines between Florida and Hatteras that were run in May. The 1933 data have been reduced and plotted, but as yet only a preliminary study has been made.

b) The Caribbean Observations.

The data from 124 stations have been corrected and turned over to Prof. A. E. Parr. This long task was greatly speeded up by the assistance of Mr. J. H. Redfield during the summer months.

c) The Seasonal Changes in the Circulation of the Gulf of Maine.

The observations from the first four cruises in the Gulf of Maine (totalling 245 stations) have been reduced and entered on the cards. Mr. Henry Sears has drawn the temperature and salinity curves. Good progress has been made in calculating the dynamic heights at the July and September stations by Mr. Stephen Lichtblau.

During the year over 5000 water samples have been analyzed for chlorine. Mr. Alfred Woodcock has carried out the greater part of this work with excellent results. The accuracy of the determinations is now very high, largely because of the special burettes which were secured from Germany last spring. Mr. Woodcock has also aided in the routine work of correcting the temperatures and depths, and has completed the finished drawings of the 1931 and 1932 sections.

STUDIES ON DISSOLVED ORGANIC SUBSTANCE AND AMMONIA IN THE SEA

ANCEL B. KEYS

The period of investigation under the auspices of the Woods Hole Oceanographic Institution was divided into two approximately equal halves: 1) A period of collaboration with Professor August Krogh on the general problem of the dissolved organic substances in the sea; 2) the continuation of this work after Professor Krogh's departure but with the major emphasis placed on the ammonia in the sea. The first period is covered in the report of Professor Krogh.

Ammonia in the sea was studied by means of a vacuum distillation technique and a new titration procedure with the distillate; this method is free from the objections which can be brought against almost all previous methods which have been applied to the problem of ammonia in the sea. It was found possible to set up the necessary apparatus on board the "Atlantis" and to carry out determinations on fresh samples of sea water during a two weeks' cruise in the Gulf of Maine.

Ammonia values ranging from 0 to 60 mgs. NH_3–N per cubic meter were found in the systematic study of 130 water samples

from 22 stations. In the shallower waters NH_3–N values from 20 to 40 mgs. were usual; at the deeper stations lower values were found with no more than traces of NH_3 in the Gulf Stream water below 150 meters. In the course of this work it was found that NH_3 disappears rapidly from sea water stored in the dark at 0 to 5°C., and the magnitude and regularity of this phenomenon were such as to cast serious doubts on the value of any ammonia estimations on samples more than a few hours old.

During the course of the summer special apparatus was constructed for the determination of free and pre-formed ammonia, organic and ammonia nitrogen, organic carbon, and for the removal of colloids by ultra-filtration.

STUDIES ON ORGANIC MATTER 1) DISSOLVED IN SEA-WATER AND 2) PRESENT IN BOTTOM SAMPLES

A. KROGH WITH THE COLLABORATION OF MARIE KROGH AND ANCEL B. KEYS

Methods for the determination of ammonia, organic carbon and nitrogen in sea-water were worked out in Copenhagen and applied to a series of samples taken on board the "Atlantis" at stations 1467–68 and sent to Copenhagen. When the apparatus was set up in Woods Hole several new difficulties were met with and much time was spent in overcoming these, so that the results of the determination proper were only partly satisfactory.

It was shown experimentally that at most a small fraction of the organic material can be present in a colloidal state, at least 90% being in true solution. The method for determination of free ammonia was successfully applied to a considerable number of samples. The carbon and nitrogen determinations gave results of the same order as the preliminary series, but several discrepancies will require a more detailed study. An experiment undertaken in collaboration with Dr. Waksman to see if bacteria could possibly utilize the dissolved organic material seemed to show some utilization after an unusual period of latency, but this again will require renewed experimentation.

On board the "Atlantis" a small number of samples were taken from the surface layer of the bottom at depths from 1000–5000 m. (stations 1427–36). These were studied microscopically when fresh and found to contain numerous fecal pellets, then preserved in a dry state and analysed in Copenhagen by combustion.

They show decreasing amounts of organic material per gm. dry

mud from station 1728 outwards, the maximum being 16.1 mg. C. and 5.0 mg. N. (station 1728) and the minimum 4.4 mg. C. and 1.7 mg. N. (station 1732). At depths of 7–15 cm. in the bottom mud the percentage of organic material is appreciably lower.

A paper on the organic material in the bottom is in preparation.

INVESTIGATIONS OF BATHYPELAGIC ZOÖPLANKTON AND EXPERIMENTS WITH CLOSING NETS

BENJAMIN B. LEAVITT

Investigations of bathypelagic zoöplankton collected by means of closing nets, were inaugurated during the summer of 1933. This work necessitated considerable experimentation with types of releasing devices and closing nets, during two trips on "Atlantis," several kinds of gear and methods being tried out and plankton collected at definite levels between thirty-two hundred meters and the surface. On the second trip, a comparison was made of the relative efficiencies of various types of two-meter canvas collared stramin and silk nets, and plankton collected for analysis in some thirty hauls at several depths at one station located east of the edge of the continental shelf some two hundred miles east of Nantucket. A new type of releasing device has been developed, and the sorting and identification of the plankton begun.

STUDIES ON THE DEFAECATION OF MARINE INVERTEBRATES

RODERICK MACDONALD

Observations were made on the defaecation of some bottom living marine invertebrates in the Woods Hole region, with results confirming in part what I have found elsewhere.

The excremental pellets, owing to their definite form, size, and in some cases sculpturing, can be identified as belonging to the following Phyla: Echinodermata, Mollusca, Vermes, and Crustacea. In some animals, the faecal pellets are so resistant to deformation that they can be identified even though overlaid by 3 or 4 inches of mud. The nature of the material taken in by the mouth appears to determine the relative resistance of the faecal pellet to disintegration.

The rate of defaecation has been studied in some bottom forms and in some cases, e.g. in *Arbacia punctata*, may be so considerable that extensive peletisation of the bottom on which the animal lives

indicates a possible influence on the relative mechanical stability of the bottom deposit.

Generally speaking, the animals studied reject their own faecal pellets, as such, as food. If, however, the faecal pellet is macerated the animal may feed on it.

Observations were also made on the faecal pellets of animals feeding on the same bottom deposits, as evidence of the degree to which different animals extract the organic matter supplied by these deposits.

PENETRATION OF RED LIGHT INTO THE SEA AND ITS RELATION TO THE PHYTOPLANKTON POPULATION

ROBERT H. OSTER

a) Measurements of the intensity of daylight in the red region of the spectrum (6000 to 7000 Ångstroms) on deck, and at depths down to 30 m. beneath the sea surface were made within the Gulf of Maine, on Georges Bank, and in the waters near Woods Hole. The photo-electric method of Poole and Atkins as used by Dr. George L. Clarke was employed in connection with a caesium on caesium oxide photocell under selective filters. Observations showed that red light was decreased to 1 per cent of its over-surface intensity at 12.5 m. in the deeper portion of the Gulf of Maine, at 11.5 m. on Georges Bank, at 10.5 m. near Gay Head, and at 7 m. in Woods Hole Harbor. It was also found that the transmissive exponent for light of these frequencies was approximately 3.5 times that for blue light (4360 Ångstroms).

b) Collections of phytoplankton were made at different depths to determine the possible relation between the intensity of the red light and the population density. Analysis of these by Mr. Trygve Braarud showed that the flagellate, *Pontosphaera Huxleyi*, was the dominant species in July 1933 at both the Gulf of Maine and Georges Bank stations, reaching its maximum at a depth of 18 meters at the former and at 12 meters at the latter station. In connection with the energy requirements of this organism it may be significant that the light intensity at both of these depths was 50.0 micro-watts per cm.^{2}

STUDIES OF FISH COLORATION

G. H. PARKER

During the season under consideration, three pieces of work were undertaken in the Oceanographic Institution. The first was on the

color changes of the smooth dogfish, *Mustelus canis*. This work was carried out with the assistance of Miss Helen Porter. The common dogfish has a dark phase and a light one due to the expansion and the contraction of its dermal melanophores. The dark phase is induced by pituitary secretion carried from the pituitary gland to the melanophores of the skin by the blood and lymph, as described by Lundstrom and Bard. The light phase was found by us to be induced through the action of contracting nerves and is not merely the result of the absence of pituitary secretions as was implied by Lundstrom and Bard. The expanding hormone is water soluble; the contracting one, if there be such, is apparently not water soluble. Mustelus is remarkable as the first fish to be described in which the cutting of nerves induces a contraction of the melanophores with a consequent lightening of the skin, not an expansion of these cells. This paper is in process of publication in the Biological Bulletin.

The second piece of work was on the identification of color changes in the Little Skate, *Raja erinacea*. This result is in contrast with Schaefer's statement that European skates of the genus Raja show no color change. This paper has been presented for publication in the Proceedings of the National Academy of Sciences.

A third investigation was begun on the color changes of certain flatfishes. This work was left incomplete with the intention of bringing it to an end in the season of 1934.

INVESTIGATIONS IN COÖPERATION WITH THE BINGHAM OCEANOGRAPHIC FOUNDATION OF YALE UNIVERSITY

A. E. PARR

Three coöperative series of investigations were started during 1933. (1) An oceanographic survey of the Central American Seas continuing the work previously started by the Bingham Foundation, (2) a quantitative and biological study of the Sargasso weeds and their associated animal life, and (3) taxonomic and distributional studies of deep sea fishes.

A jointly-sponsored cruise with the "Atlantis" made 132 oceanographic stations in the Caribbean area, at which about 2000 observations of temperatures and oxygen were made and a corresponding number of sea water samples were collected for subsequent determination of salinities. The titration and calculation of salinities and oxygen, the correction of temperature and estimation of depths

of observation from the readings of the unprotected thermometers were all completed during the year by the laboratory staff of the Institution, and the data thus prepared for further analysis, now in progress.

By the towing of a specially designed surface dredge continuously over long distances, a first attempt was made to obtain an approximate idea of the actual amount and the quantitative significance of the floating Sargasso weeds. The dredge proved successful for use with the ship progressing on its course at its normal speed and was towed for about 260 miles altogether during the Caribbean cruise. The weights per mile of the various species of weeds thus collected were recorded and samples preserved for further study of the taxonomy and ecology of the weeds and of the associated animal life.

During the Caribbean cruise, several attempts were also made to employ in deep sea collecting a triangular trawl measuring 50 feet along each side of the opening. While mechanical difficulties which can be easily overcome by a few simple installations on board impeded the work during the first experimental tryouts with this large gear, the results of one of the successful hauls adequately proves the value of a large net of this type for obtaining information about the larger and faster species of deep sea animals. No less than 47 separate forms of deep sea fishes alone, and among these 6 new species and subspecies, were obtained in this single catch, and the number of specimens of significantly large size is more than three times the average number of such specimens obtained in 18 hauls with a 14-foot circular net in the same general region. A report on these deep sea fishes is now ready for publication.

Identification of certain groups of deep sea fishes in the earlier "Atlantis" collections was also carried on.

STUDIES ON THE CHEMISTRY OF SEA WATER

NORRIS W. RAKESTRAW

In the chemical laboratory of the Institution work on several problems was carried out during the past summer.

Perhaps the most important of these was the beginning of an intensive chemical survey of the Gulf of Maine, to be continued throughout the present year, in the effort to get data covering the seasonal variations of phosphate, nitrate, nitrite, pH, and possibly buffer capacity, at a large number of stations in the Gulf.

Chemists from the laboratory accompanied the "Atlantis" on

most of her trips from June to October, and in addition to the routine analyses just mentioned, samples were brought back for work on boron, iron, copper, and for other purposes.

The study of the significance of nitrite in the nitrogen cycle in the sea, and of its distribution and normal variations, was carried to a further stage.

Samples from a dozen stations were analyzed to determine their boron content, in order to compare the results from North Atlantic water with those obtained from the Pacific at the Scripps Institution. Our results were in general agreement with theirs, with only slight differences in the ratios found between boron and chlorine.

We think we have almost completely realized the hope we have had for two years of obtaining an accurate measurement of the concentration of free, inorganic iron in the sea water. Many difficulties were encountered in the analytical procedures and with each improvement in technique the amount was shown to be less. While we think our eventual figures are fairly accurate we need analyses on more samples before they can be considered conclusive.

A start was made on what we hope to be a large collection of the ash residues of various marine organisms. All this material will eventually be investigated spectroscopically in a systematic study of its inorganic constituents.

Some progress was made on two problems with which we had hoped to get somewhat further: namely, the analytical determination of some of the heavy metals, particularly copper; and the application of spectroscopic and photo-electric analytical methods. The unexpectedly large amount of time devoted to the field work of the summer prevented further progress in these directions.

STUDIES IN MARINE BIOLOGY AND BIOLOGICAL CHEMISTRY

ALFRED C. REDFIELD

During a cruise of the "Atlantis" in February data were obtained, through the coöperation of Dr. Rakestraw, which have been incorporated into a paper entitled "On the proportions of organic derivatives in sea water and their relation to the composition of plankton" to appear in the James Johnstone Memorial Volume. The principal conclusion drawn in this paper is that the proportions in which the quantities of nitrate, carbonate, phosphate, and oxygen occur in different samples of water from the open ocean are governed strictly by the composition and quantity of plankton which have decomposed

in the water in question, and that uniform conditions in this regard obtain in the various oceans of the world.

The systematic investigation of the waters of the Gulf of Maine, mentioned on page 17, was carried on with cruises in June, September, October, December, and January and is to be continued during the spring with a view to obtaining an accurate picture of the distribution of substances important in the nutrition of animals and plants throughout the year within this peculiarly isolated body of water. It is hoped that these measurements will serve to correlate the data on the distribution of plankton within the Gulf and the circulation of its waters, already known in their general outline from the work of Bigelow. Analysis of the data secured to date indicates that the deficiency of oxygen in the deeper waters of the Gulf, noted in last year's report, is due primarily to the condition of the water at the time of its entry into the Gulf. There appears to be, however, a considerable additional loss of oxygen by the deep water after it has entered the Gulf, particularly in the western basin. The extensive systematic series of hydrographical data, secured during these cruises, promises to provide the basis of hydrographical calculations and other estimates which will give a much more detailed picture of the actual circulation within the Gulf than we have at present, a picture which is indispensable to the interpretation of the chemical and biological data.

Dr. R. W. Root and Dr. Arda A. Green have continued their studies on the hemoglobin of fishes. The work this summer, dealing with the equilibrium between hemoglobin and carbon monoxide, was designed to elucidate certain theoretical questions arising from last year's studies. Dr. Root has completed an investigation of the combination of hemocyanin with carbon monoxide and has demonstrated for the first time without doubt that such combinations occur. Mr. Walter Buck gave Doctors Root and Green valuable technical assistance during the summer.

INVESTIGATIONS IN METEOROLOGY AND PHYSICAL OCEANOGRAPHY

C. G. ROSSBY

During the past year the theoretical investigation of the homogeneous surface layer of the ocean was continued. An attempt was made to study the effect on converging and diverging flow on its thickness. Empirical data from a number of oceanographic cruises were tabulated for a test of the theoretical conclusions. Work has

been done on the development of the baro-thermograph for accurate recording of the vertical temperature distribution within the surface layer (0–200 m.). This instrument, which has been tested in the Hydraulics laboratory at the Massachusetts Institute of Technology, is now practically completed. It is based on the same principles as the Jaumnotte sounding balloon meteorograph.

Mr. Lichtblau has completed the routine evaluation and tabulation of all evaporation measurements made on board the "Atlantis," but no analysis of this material has been attempted.

Two separate dynamic calculations of the circulation in the Gulf of Maine were made by Mr. Lichtblau.

ZOÖPLANKTON STUDIES

MARY SEARS

1. *The distribution of the zoöplankton on the continental shelf, Cape Cod to Chesapeake Bay.*

During the past summer, some 150 samples of the May-June 1932 catches of plankton were identified and sorted for a quantitative estimate of their constituents, with the aid of Miss Catherine Goffin. There now remain but a few samples of the May 1930 "Albatross" cruise, some of those from the February 1932 "Albatross" cruise, all those from the October 1931 "Atlantis" cruise, and some from the May-June 1932 "Albatross" cruises, to examine before proceeding with the report.

2. *Siphonophores collected on the "Thor" expedition to the Mediterranean, 1908 and 1910.*

The preliminary examination and counting of the collection has been completed. Specimens which could not be readily referred to the common species were set aside for further study, which was started during the summer. There still remain some mutilated specimens of certain Galettas and odd nectophores of some of the Vogtias to be examined.

CHEMICAL STUDIES OF PLANKTON IN SEA WATER

GLADYS E. SEIWELL

Chemical studies on the organic carbon and phosphorus content of marine plankton were undertaken with a view to calculation of organic production, based on theoretical calculation of oxygen and phosphorous consumption in the tropical Atlantic ocean. Carbon

was determined according to Liebig's method, by burning the substance in oxygen and collecting and weighing the carbon dioxide. Phosphorus was determined by modifying an alkalimetric method in which the phosphomolybdate precipitate was dissolved in standard alkali and the excess titrated with standard acid. The phosphorus determinations were made in triplicate and the agreement of results is good. For seventeen samples of deep sea plankton carbon (C) varied 28 to 45 per cent and phosphorus (P) 0.5 to 1.5 per cent. A report of the work is in preparation.

A comparison of several methods for determination of iron in sea water was undertaken in preparation for a study of the iron content and consumption of iron by the phytoplankton. Methods used at the Institut für Meereskunde in Berlin, and in Professor Hjort's laboratory at the University of Oslo were tried. A method making use of the thiocyanate reaction appears to be the most satisfactory. The results appear to be good and the colors definite and readily comparable. To date total iron has been determined for several stations made on cruise 23 of "Atlantis."

During the year a paper, "Über den Gesamtphosphorgehalt des Seewassers im westlichen Nord Atlantischen Ozean" has been prepared in collaboration with H. R. Seiwell. This paper is based on soluble phosphorus observations obtained during cruise 6 of "Atlantis" and total phosphorus observations made in the laboratory at Woods Hole on samples collected during the same cruise.

STUDIES OF PHOSPHORUS AND OXYGEN CONTENT OF WESTERN NORTH ATLANTIC BASIN

H. R. SEIWELL

A report on the oxygen content of the western North Atlantic basin is nearly completed. This discussion is based on the oxygen observations made by "Atlantis" during three years of cruising in the area together with the oxygen results of earlier expeditions. The report includes a descriptive discussion of the horizontal and vertical variations of oxygen, the seasonal variations, and a theoretical discussion and calculation of the annual oxygen consumption and its relation to biochemical activity.

A report on the nutrient phosphorus content of the western North Atlantic basin is well advanced. This report includes a descriptive discussion of the phosphorus content of the region, the seasonal variation, and a theoretical discussion of the phosphorus-oxygen relationships in relation to the chemical fertility of the region.

During the year two other papers were prepared for publication.

1. *Über den Gesamtphosphorgehalt des Seewassers im westlichen Nord Atlantischen Ozean* (by H. R. Seiwell and G. E. Seiwell) is in press. This paper is based on total phosphorus and soluble phosphorus observations in the western North Atlantic made during cruise 6 of the "Atlantis."

2. *The Production of Organic Matter in the Tropical North Atlantic Ocean.* This discussion includes a theoretical calculation of the annual production of organic matter based on the results obtained by studies of the content, consumption and production of oxygen and phosphate phosphorus in the region.

PHYSICAL OCEANOGRAPHY OF THE GRAND BANKS REGION FROM DATA COLLECTED BY THE INTERNATIONAL ICE PATROL IN THE SPRING AND SUMMER OF 1933

FLOYD M. SOULE

During the Ice Patrol season of 1933 six cruises were made in the vicinity of the Grand Banks of Newfoundland, and on each cruise data were collected for the construction of surface isothermal charts of the region. During this period 25 stations were occupied, at each of which temperatures and salinities were measured from the surface down to 1,000 meters where the depth permitted. The results of this work are being published in U. S. Coast Guard Bulletin No. 23 which is now in press.

A post-season northern cruise was made in the waters between Newfoundland and Labrador, and southwest Greenland between June 26 and July 24 during which 112 stations were taken, most of them being reoccupations of stations taken on the 1931 northern cruise. At these stations temperature and salinity measurements extended from the surface down to 2,000 or 2,500 meters where the depth permitted. Arsuk glacier in southwest Greenland was visited and photographs recording its retreat were made. The dynamic calculations have been completed and horizontal and vertical sections of temperature, salinity, and density are now being prepared.

In the Grand Banks region, chiefly in the rectangle between 46° and 48° N. Lat., and between 45° and 48° W. Long., 128 fathometer soundings were taken, and 594 more on the northern post-season

cruise. These figures apply only to soundings for which positions have been determined and for which correction data are available.

REPORT ON STUDIES IN SEDIMENTATION AND SEDIMENTARY ENVIRONMENTS

H. C. STETSON, MARSHALL SCHALK, C. W. TAYLOR

1. During the winter "Atlantis" made four traverses across the continental shelf on which bottom samples were obtained. Starting from the shore at the following places, Onslow Bay, S. C., Charleston, S. C., Jacksonville, Fla., and Cape Canaveral, Fla., the lines were carried down the slope and into deep water under the Gulf Stream. This completes the traverses, eleven in all, from Cape Cod to Florida, which were originally planned as a survey of the sediments of the shelf. The data have all been compiled and a general report is in progress.

2. The bottom cores obtained in the Arctic Basin by the "Nautilus" were analyzed and the report published.

3. Direct observations on the rates of transportation and deposition were more successful this year than last, thanks to our improved type of sedimentation pan. Nine stations were made in shoal water with individual sets varying from a week to ten days. Three of these sets were made in Massachusetts Bay during November and December. The observations, meagre as they are, emphasize how theoretical is our knowledge of this phase of marine activity.

4. It is becoming increasingly apparent that a detailed study must be made of the sediments of several small water-bodies in which the geological history of the factors affecting sedimentation is neither long nor complicated. By this means certain principles may be established which can be applied to the interpretation of more complex areas, such as the continental shelf. Cape Cod Bay is ideal for such a study, with Massachusetts Bay and Vineyard and Nantucket Sounds supplementing it in somewhat lesser degree. Accordingly work was started in the sounds last summer and considerable dredging done in Massachusetts Bay this fall. Mr. Schalk is now carrying out the mechanical analysis of these samples.

5. As an approach to the study of the part played by the beach cycle in marine sedimentation, including the effects of wave action and lateral travel along the beach, a suite of sand samples was collected on Cape Cod, from Long Point at Provincetown to the end of Monomoy Point, and from Cape Poge to Gay Head along the southern shore of Martha's Vineyard. The station interval was gen-

erally one half mile, with samples taken at high, and either mid or low tide. The samples are now being studied in the laboratory by mechanical analysis and mineralogic variation. A report is in progress.

STUDIES ON THE COLLOID OSMOTIC PRESSURE OF THE BLOOD OF FISHES

ABBY H. TURNER

In the summer of 1933 the colloid osmotic pressure and refractometric index of the serum was determined in varying numbers of individuals of 5 species of teleost and 3 species of elasmobranch fishes. Though the number of determinations was not large, owing to the short time available for work, the following generalizations seem justified.

1. The colloid osmotic pressure of elasmobranch blood is much lower than that of teleosts, an average of 41 mm. water pressure as compared with 139 mm. This does not seem out of harmony with the pictures of elasmobranch and teleost blood as given from other points of view by Homer Smith.

2. The different species of teleosts are characterized by individual ranges of colloid osmotic pressure, averaging as follows:

Paralichthys dentatus	summer flounder	178 mm.
Prionotus carolinus	common sea robin	86 "
Prionotus evolans strigatus	red-winged sea robin	92 "
Sarda sarda	bonito	233 "
Tautoga onitis	tautog	148 "

Whether these different values are associated with differences in life habits or with other factors is at present undetermined.

3. Single studies on two invertebrates, Loligo and Limulus, showed values, 84 and 63 mm. respectively, not out of the general range of the fishes.

INVESTIGATIONS IN MARINE BACTERIOLOGY

SELMAN A. WAKSMAN, H. W. REUSZER, C. L. CAREY, M. HOTCHKISS, W. F. BRUCE AND C. E. RENN

The investigations in the field of Marine Bacteriology carried out at the Institution during 1933 were to a large extent continuations of those of 1932, namely, 1) the abundance and distribution of the bacterial population in the sea, 2) decomposition of algal residues by bacteria, 3) the rôle of bacteria in the nitrogen cycle of the sea, es-

pecially in nitrification and nitrogen-fixation, 4) the abundance and chemical nature of marine humus in the sea bottom. The first and last of these investigations approach completion. Two new problems were begun: 1) the origin and chemical nature of the organic matter in the sea water, and 2) the rôle of bacteria in the disappearance of Zostera along the Atlantic coast. The results of these investigations can be briefly summarized as follows:

The distribution of bacteria in the sea is chiefly limited to three centers or loci of bacterial life: 1) The marine plankton, with unequal distribution of the bacteria within the animal and plant plankton. 2) The marine bottom, with a marked difference in the abundance of bacteria in sand and mud bottoms. 3) The sea water itself, which represents, however, only a poor medium for bacterial development, as compared with the other two loci. Sea water in shallow regions, where the water tends to be mixed from top to bottom, contains much larger numbers of bacteria. This can be correlated with the greater plankton development and the greater amount of organic matter made available to the bacteria. The horizontal distribution of bacteria in the extreme surface layer of mud is independent of the amount of organic matter present in the mud but is largely dependent upon the degree of its decomposition; below the surface layers of mud, the distribution of bacteria is directly correlated with the organic matter content. The bacterial numbers on the continental shelf were found to decrease regularly with distance from land; however, in the deeper layers of mud, the numbers remained constant.

The numbers of bacteria obtained by the plate method were found to represent only a small fraction of the total bacterial population of the sea, probably about 0.5 per cent, as determined by direct microscopic analysis.

A number of bacteria were isolated capable of decomposing alginic acid, the polyuronide carbohydrate present abundantly in marine algae. These bacteria were studied in detail and their description will necessitate the creation of several new species. In the decomposition of alginic acid in sea water media, these bacteria produce enzymes which hydrolyze this complex to smaller groups, but not to simple mannuronic acid. In sodium chloride media, however, these bacteria produce from alginic acid reducing substances (mannuronic acid?) and a very extensive gel which is sufficient to change the whole medium originally liquid into a thick jelly-like mass. The nature of the gel and its rôle in the cycle of organic matter in the sea is being investigated.

It was also found that in the decomposition of the algal material

by these bacteria, a complex is formed, high in carbon (62 per cent) and in nitrogen (3.5–4.0 per cent) which readily dissolves in a slightly alkaline solution. This complex is resistant to decomposition by bacteria.

As to the study of bacteria concerned in the nitrogen cycle, the following findings may be reported: 1) the presence of nitrifying bacteria in the sea bottom has been established for ocean depths of 4,000 to 5,000 meters. 2) The presence of anaerobic nitrogen-fixing bacteria of the Clostridium type in the sea. 3) The ability of Azotobacter isolated from the sea to fix considerable quantities of nitrogen.

In connection with the investigation of the disappearance of the *Zostera marina*, several species of bacteria were isolated from the dead plants. Attempts have been made to bring about the disease under controlled laboratory conditions. In view of the specific nature of the Zostera plant and the difficulty in cultivating it under controlled conditions, much time had to be spent in developing suitable methods. A number of photomicrographs have been made of sections of the Zostera illustrating the progress of the disease. It is hoped that with the preliminary studies completed, definite progress can be made during the next growing season.

HYDROGRAPHY OF THE BAY OF FUNDY

E. E. WATSON

The investigation of the International Passamaquoddy Fisheries Commission which terminated in the spring of 1933 left a considerable quantity of hydrographic material to be examined in further detail. During the summer of 1933 this material was analysed and when possible correlated with Mr. Braarud's phytoplankton data for the same period. As the observations were made in a single summer over the whole Bay of Fundy they are necessarily inadequate for a detailed picture of the water movements, but they give the best record of the seasonal progress that has yet been obtained in this region. The oscillatory motion of the tide along the trough of the Bay of Fundy is complicated at the mouth by Grand Manan I. and its banks and shoals. These produce a complex of vortices which vary so rapidly in place and time that deductions from individual sets of observations frequently disagree. It is only possible to draw a picture which is true in general though seldom in detail.

The final hydrographic report on this work is now in process of preparation for publication and should be completed in 1934.

STUDIES ON COPEPODS

C. B. WILSON

The identification and recording of the planktonic copepods captured during the last cruise of the "Carnegie" has been completed. Lists of species have been compiled for each of the 200 localities in the Atlantic Ocean north of the equator and in the Pacific Ocean from 52° north to 40° south latitude. Data with reference to the temperature, salinity, density, hydrogen ions and phosphates of the ocean water for each of the depths at which tows were taken have been included in each of the lists.

These are to be deposited with the Carnegie Institution at Washington, D. C.

A good start was made in the study of an exceptional towing taken from "Atlantis" with a closing net at a depth of 2000 meters south of Georges Bank. Twenty-five genera, including about 35 species, of copepods were identified, showing the presence of a considerable copepod community even at so great a depth.

Finally a series of 17 copepod towings from the vicinity of the Bermuda Islands, containing about 150 species, were identified for the U. S. National Museum.

List of Contributions

LIST OF CONTRIBUTIONS

FROM THE WOODS HOLE OCEANOGRAPHIC INSTITUTION

No. 1. H. U. Sverdrup. Scientific Results of the *Nautilus* Expedition, 1931, under the Command of Captain Sir Hubert Wilkins. I. Introduction and Narrative. *Papers in Physical Oceanography and Meteorology*, Vol. II, No. 1, pp. 3-15.

No. 2. H. U. Sverdrup. Scientific Results of the *Nautilus* Expedition, 1931, under the Command of Captain Sir Hubert Wilkins. II. Oceanography. *Papers in Physical Oceanography and Meteorology*, Vol. II, No. 1, pp. 16-63.

No. 3. Floyd M. Soule. Scientific Results of the *Nautilus* Expedition, 1931, under the Command of Captain Sir Hubert Wilkins. III. Echo-Sounding. *Papers in Physical Oceanography and Meteorology*, Vol. II, No. 1, pp. 65-76.

No. 4. B. Villinger. Scientific Results of the *Nautilus* Expedition, 1931, under the Command of Captain Sir Hubert Wilkins. IV. Die Schweremessungen auf der *Nautilus* Expedition, 1931. *Papers in Physical Oceanography and Meteorology*, Vol. II, No. 3, pp. 3-16.

No. 5. Henry C. Stetson. Scientific Results of the *Nautilus* Expedition, 1931, under the Command of Captain Sir Hubert Wilkins. V. The Bottom Deposits. *Papers in Physical Oceanography and Meteorology*, Vol. II, No. 3, pp. 17-37. 1933.

No. 6. Selman A. Waksman and Cornelia L. Carey. Rôle of Bacteria in the Decomposition of Plant and Animal Residues in the Ocean. *Proc. Soc. Exper. Biol. and Med.*, Vol. XXX, pp. 526-527. 1933.

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